CEREAL CHEMISTRY

Vol. VIII. January, 1931 No. 1

A STUDY OF SOME METHODS OF EXAMINING FLOUR, WITH SPECIAL REFERENCE TO THE EFFECTS OF HEAT. I. EFFECTS OF HEAT ON FLOUR PROTEINS [1]

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(Received for publication June 12, 1930)

Earlier researches were concerned with the relationship between the chemical constituents of flour and its "strength" or baking quality. Crude gluten, total protein and the glutenin-gliadin ratio have variously been cited as the predominating factor in determining this property of flour. Woodman (1922) has suggested that the constitution of the glutenin may not be fixed and that this variable is of importance, although his point of view has not generally been accepted. The fat and lipoid contents have also been investigated with a view to determining their effects on the baking quality. Working (1924) believes that the phosphatide content plays an important role in bread doughs.

Little progress was made from the chemical standpoint in the formation of a clear conception of the meaning of baking quality, but the "quality" of the gluten has been associated frequently with the ideas of strength in flour; this paved the way for a physico-chemical theory. Wood (1907) and Wood and Hardy (1908) were among the first to emphasize that baking quality might be more related to the physical properties of the proteins than to the chemical composition. Upson and Calvin (1915) held similar views and the work was continued by Gortner and Doherty (1918), who applied the conception of the colloidal state in order to explain the differences in baking strength; they regarded gluten as a colloidal gel, whose physico-chemical properties varied from flour to flour. The works of Ostwald (1919) and Mohs (1922) are well known in this connection. These studies naturally led to the attempt to measure the colloidal condition of a flour, and in view of Graham's work on the viscosity of colloidal solutions, this property was one

[1] Condensed from a Thesis approved for the Degree of Doctor of Philosophy in the University of London.

of the first to receive attention. As a result of this work, the view is now generally held that the quality of the protein is one of the main factors controlling baking properties and that this "quality" factor is determined largely by the imbibitional power of the proteins, i.e., the colloidal condition.

Swanson (1925) has given a very clear picture of this theory of colloidal behaviour; he states:

> "We have at least two continuous phases, water and the gluten meshwork. In this double continuous phase system are held the starch grains as well as the non-gluten proteins, all covered by a film of water. In this water are found molecularly dispersed salt, sugar and simpler organic compounds such as amino-acids or soluble proteins. . . . Into this most complex environment we place the yeast plant. When we make a baking test we thus deal with physical, chemical and biological forces, and the baking test becomes one of the most delicate tests we can imagine."

Baking quality will depend, therefore, as one essential upon the colloidal dough structure and upon the strength of the fibrils of the protein meshwork, and these in turn are determined by the colloidal condition of the protein particles in the original flour. The strength of these fibrils will depend to some extent also on the number of protein particles, i.e., on the amount of protein present.

Given the acceptance of the colloidal theory of dough structure and the knowledge of the sensitiveness of colloids to the effects of heat, it is little wonder that attention was directed to attempts to change the state of the proteins by heating. The use of heat has been discussed with regard to the conditioning process, i.e., to get the endosperm and the outer coatings of the berry into a condition of easy separation, by Tague (1920), Simon (1923), and Kirby (1923), but Kent-Jones (1926) was the first deliberately to apply heat with the object of changing the colloidal condition of the proteins. Kent-Jones in 1924 was granted British Patents Nos. 228,829, 228,830, 228,841 and 263,897 for the commercial exploitation of the process, and, subsequently, patents for similar heat treatment processes have been granted to Fisher and Jones in 1928 (British Patents Nos. 300,291 and 300,537), Noury and van der Lande in 1928 (British Patent No. 300,568) and Hall and George in 1929 (British Patent No. 310,125). On the scientific side, however, the work of Kent-Jones (1926) stands alone as the original and fundamental contribution to our knowledge of the changes taking place during the heating of wheat and flour. Kent-Jones

could detect no effect on the strength of a wheat by the normal conditioning process; his opinion is that the conditioning improves the milling properties of the wheat and thereby indirectly the flour also. More severe heating, however, under certain conditions, markedly enhances the baking quality; there is in the first period no noticeable improvement in baking strength, followed by a definite period of maximum increase and then continued heating ruins the wheat or flour from a baking point of view; if this "overheated" flour or wheat be mixed with the unheated sample, there is a definite improvement in the dough behaviour. The quality of the gluten is changed by this heating; at the correct time for maximum improvement there is a toughening but afterwards the gluten becomes "short" and snappy and loses its power of cohesion, so much so that eventually the gluten cannot be retained in a washing test.

A detailed study of these heated and overheated flours showed that there was a definite increase in the relative viscosity of the water suspension over that of the untreated; heating reduced the amount of protein soluble in 5% potassium sulphate solution and the solubility of the albumen in water. Kent-Jones used the fall in viscosity of flour suspensions kept at 27° C. to indicate the proteolytic activities of the flour, and concluded therefrom that proteolytic activity is not proportional to weakness, for some strong flours have quite marked proteolytic activities, while many weak flours are lacking in this.

Heating is suggested as a means of increasing the degree of aggregation of the proteins, so that the imbibitional power is augmented and consequently increased strength of the dough structure is obtained. With regard to the improving action of the added "overheated" flour, the amount of which by suitable times and temperatures can be reduced to as low as one per cent, Kent-Jones (1926, p. 107) says:

> "There is little doubt that the increased baking value is due chiefly to the increased imbibitional power of the heated proteins. . . . At the commencement of fermentation it has little, if any, effect. Then as the dough wants to slacken out, this abnormally swollen nucleus knits the whole together. Usually the longer the fermentation, the more the slackening of the dough and the feeling of weakness; at the same time the more this nucleus is swelling and stiffening the otherwise too soft dough. Such overheated flour thus becomes an 'improver' of a very special and valuable nature."

Berliner and Rüter (1928a) have considered Kent-Jones's work in some detail and have extended the observations on the effect of heat upon flour. They confirmed the toughening effect on the gluten, until the stage was reached when no gluten was retained, and also found that the higher the water content of the wheat or flour, the more sensitive it is to the effects of heating. They claim that if flour which has been previously dried in a vacuum at room temperature be heated for 15 hours at 80° C. in an open vessel, the gluten is not damaged; that is, if the majority of the water is removed at a low temperature, then the irreversible coagulation of the gluten does not take place.

Kent-Jones' relative viscosity measurements during incubation are criticized in that the bactericidal solution used by him (1 : 10,000 auramine) is not effective, and that, therefore, in these experiments there is an interfering factor in the acid swelling of the flour particles caused by the growth of bacteria. Berliner and Rüter suggest the use of a 0.05N lactic acid solution for making the suspension; this has a pH less than 3.0, at which they state there is no appreciable development of bacteria. The viscosity changes are, however, of a similar nature to those of Kent-Jones.

A very interesting observation of Berliner and Rüter was that the strongly overheated flour appeared distinctly different from the unheated when viewed under ultra-violet light; according to them the former shines up light blue between untreated or only lightly treated flours. Kent-Jones (1928) in a reply to Berliner and Rüter suggested that this effect may be due to the granular nature of the material and not specifically to the effects of heat.

It is essential in this resumé to consider the methods of measuring baking strength, or baking quality. The fundamental and final test is undoubtedly the baking test itself, but some numerical record of this is desirable for satisfactory comparisons. The first thing the experienced baker studies is the behavior of the dough during fermentation and this is followed very carefully from the making of the dough right through to the final proof, when it is put into the oven; afterwards there is the appearance and build of the loaf to be considered, as well as the crumb texture. It is in this recording of the baking behaviour that difficulties arise. It is argued by some workers that the final loaf is the marketed article, and, therefore, that should be the sole criterion of the successful behaviour of the dough; accordingly it has become popular, particularly in America, to measure the baking quality of a flour by the volume of the baked loaf; this is done by some

method of displacement. Other schemes involve the "scoring" of bread, in which numerical figures are allotted in respect of color, texture and spring of the crumb, as well as volume. Some workers, however, notably Kent-Jones, do not regard the loaf volume as a satisfactory indication of baking quality; he judges baking strength by the general behaviour, firmness, stability, spring, etc., of the dough during fermentation and this is concerned with the quantity and more particularly the quality of the gluten proteins. He then bakes cottage bread, which in general will give a bolder and better upstanding loaf with a stronger flour.

Berliner and Rüter (1928a) failed to observe the same improvement in baking quality due to the effect of heat and overheated flours. They refer to the fact that Kent-Jones nowhere gives exact numerical details of the improvements in baking which he obtained, and state:

> "We must, therefore, record that both with the original K-J flour and with additions of heated flour prepared in accordance with the instructions given, we have found indeed a certain improvement in the stability of weak doughs and perhaps also a somewhat better standing up of cottage loaves, but that these differences in the quality of dough and loaf were so small that for the most part they did not show themselves at all clearly, to say nothing of their being capable of measurement."

The criticism of the loaf volume measurement method is undoubtedly sound, but for comparative purposes the judgment of the fermenting dough has a great disadvantage in that no numerical record is obtained; this method is, however, probably more in accord with the commercial baker's method of judging the baking quality of the flour delivered to him.

It is natural, therefore, to look for some method of recording the properties of a dough; several suggestions have been made in the literature. Among the more important may be mentioned Jago (1911), Bailey and Le Vesconte (1924), Kress (1924), and the penetrometers of Harrel (1927) and Reinders and van der Lee (Kent-Jones, 1927, pp. 145-147). St. John and Bailey (1929) used three devices to test the effect of dry skim milk upon the water absorption of doughs and in central Europe the apparatus of Hankóczy is well known. The author has not had an opportunity of examining all these instruments, but is of the opinion that a satisfactory method of recording dough properties would be of

great value in the study and measurement of baking quality or strength.

The object of the present work was to extend the investigations begun by Kent-Jones, to consider the criticisms of Berliner and Rüter, and, further, to examine a number of methods of testing the changes, which might account for the alteration in baking quality consequent upon the application of heat.

Experimental

Preparation of Material.—As a basis for this study portions of a bag of No. 3 Manitoba patent flour were heated under conditions laid down in the Kent-Jones patents for various times and temperatures; thus:

No. 1. Untreated.
No. 2. Heated 11 hours at 135°F.
No. 3. Heated 2 hours at 150°F.
No. 4. Heated 1 hour at 160°F.
No. 5. Heated 40 minutes at 170°F.
No. 6. Commercial "K-J flour", i.e., heated 10 hours at 180°F.

After heating, they were removed from the containing vessel, cooled and dressed. Examination of the doughs during a baking test indicated that samples Nos. 2, 3, 4 and 5 were distinctly stronger than No. 1; the doughs stood firmer and bolder and had greater stability, fully in accord with the findings of Kent-Jones. The addition of 0.7% of No. 6 to No. 1 produced very similar improvement results, and the dough qualities were markedly superior to those of the untreated throughout fermentation. The treated loaves were all bolder than the untreated.

Imbibitional Powers.—Many attempts have been made to use viscosity determinations as an index of flour strength; among these may be mentioned the works of Ostwald and Lüers (1919) and Sharp and Gortner (1922). These have not been entirely successful, due, probably, to the fact that baking quality is a function of many variables. Further, the viscosity determination is one which is dependent upon external conditions and can easily be affected by impurities (e.g. bran powder) and by methods of manipulation, as shown by Johnson (1927). It is probable, however, that in one and the same flour, changes in the physical condition of the protein and its imbibitional powers can be recorded viscometrically, and that in this viscosity determination there exists a very useful method of studying the effects of heat upon the flour proteins. Kent-Jones (1926) and the present writer have used the viscosity in this way. The specially constructed Ostwald tubes used by Kent-Jones have been employed again in this work, and

the relative viscosities only have been given. The viscosity ratios may not be strictly accurate, but in view of the limitations which have to be placed on this test, it is thought that they give as much information as when calculated to true viscosity data.

The observation of Kent-Jones was confirmed that the viscosity of an untreated flour suspension in 1 : 10,000 auramine solution maintained at 27° C. increased at first and then decreased; the correctly heated flour suspensions had higher viscosities throughout but behaved similarly. The viscosity of the overheated flour suspension, however, increased rapidly as long as incubation proceeded. It was found that the H-ion concentration increased during incubation (e.g. from pH 6.0 to 3.5 in 72 hours) and, further, that there was considerable bacterial growth, as stated by Berliner and Rüter (1928a). Blood heat organisms increased in one case from 2,000 per gram to 5,000,000 per gram after 24 hours' incubation. A further experiment showed that this phenomenal viscosity increase on the overheated flour suspension was not obtained if the acidity was neutralized as it was formed, although bacterial growth proceeded in a similar way.

Berliner and Rüter suggested the use of 0.05N lactic acid in making the solutions, so that they had a pH of approximately 3.0; this gives viscosity curves of an entirely different nature and, moreover, it was found that bacterial development was retarded but not inhibited. A typical count showed an increase in blood heat organisms in 48 hours from 2,000 to 700,000 per gram.

Such a reconsideration of previous work showed that it was imperative:

(a) to find a solution which would inhibit the bacterial development completely, and

(b) to study the relationship of viscosity to the acidity of the medium of the suspension.

Many failures were encountered in the search for a satisfactory bactericidal medium, but eventually two were found, which gave fairly good results. These were:

(i) an ammonium fluoride solution of 1% strength

(ii) a stronger lactic acid solution at about a pH of 1.0

The former gave suspensions of pH approximately 4.0-4.5, whereas the latter dealt with the acid suspensions.

Tables I and II show the viscosity changes of the flour suspensions maintained at 27° C. in the presence of 1 : 10,000 auramine and 1% ammonium fluoride. Graphical representation of three typical flours is shown in Figs. 1 and 2. A comparison indicates that

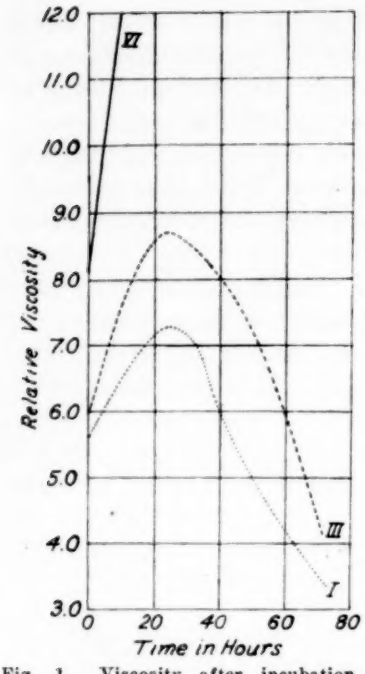

Fig. 1. Viscosity after incubation in presence of 1/10,000 Auramine. Flours I, III and VI.

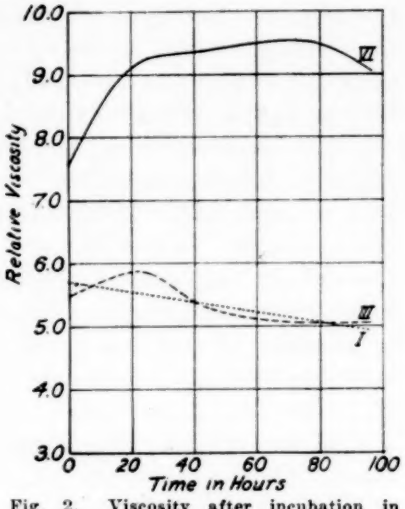

Fig. 2. Viscosity after incubation in presence of 1% ammonium acid fluoride. Flours I, III and VI.

TABLE I

VISCOSITY AND pH AFTER INCUBATION IN PRESENCE OF 1:10,000 AURAMINE

Flour	Original		After 24 hrs.		After 48 hrs.		After 72 hrs.	
	Viscosity	pH	Viscosity	pH	Viscosity	pH	Viscosity	pH
I (Untreated)	5.62	6.15	7.29	5.85	5.15	3.95	3.40	3.60
II (Heated)	6.72	6.05	8.82	5.75	7.26	4.65	4.74	4.50
III (Heated)	5.98	6.00	8.71	5.55	7.40	4.50	4.13	3.85
IV (Heated)	5.57	5.95	8.12	5.50	5.71	3.60	5.21	3.50
V (Heated)	5.72	6.00	8.66	5.50	6.39	3.70	5.46	3.60
VI (Strongly overheated)	8.12	5.95	27.00	5.50	52.30	3.65	63.10	3.65

TABLE II

VISCOSITY AND pH AFTER INCUBATION IN PRESENCE OF 1% AMMONIUM FLUORIDE

Flour	Original		After 24 hrs.		After 42 hrs.		After 72 hrs.		After 96 hrs.	
	Viscosity	pH	Viscosity	pH	Viscosity	pH	Viscosity	pH	Viscosity	pH
I (Untreated)	5.70	4.50	5.53	4.25	5.32	4.50	5.12	4.75	4.92	4.90
II (Heated)	6.45	3.95	6.31	3.95	5.86	4.45	5.49	4.70	5.17	5.00
III (Heated)	5.47	3.80	5.86	4.10	5.32	4.45	5.05	4.70	5.05	5.00
IV (Heated)	5.53	3.90	5.68	4.00	5.07	4.45	4.84	4.70	4.58	4.85
V (Heated)	5.40	4.60	5.68	4.15	5.30	4.50	5.00	4.75	4.88	4.85
VI (Strongly overheated)	7.60	4.60	9.24	4.25	9.34	4.50	9.54	4.80	9.05	5.00

in the absence of bacterial growth and at a pH 4.0-5.0, the viscosity decrease of untreated and normally treated flours is small, but that the tremendous increase in the viscosity of the overheated flour is not obtained. The tremendous importance of bacteria in an incubating suspension is hereby emphasized, particularly when dealing with heated flours; in all work it is essential to know exactly whether or not bacterial growth has taken place.

With the lactic acid (pH 1.0) suspensions, the original viscosities were very much higher than previously and there was in general a slight increase on incubation. The viscosity of the strongly overheated flour was enormous throughout. In this case a different set of phenomena are being dealt with (namely the "acid" swelling) but again the decrease on incubation is not large when the growth of bacteria is prevented. It appears, therefore, that the effect of heat in the first place is to increase the imbibitional power of the proteins of flour; very severe heat treatment causes a more radical change to take place with the result that a slow but remarkably great power of swelling is obtained on incubation. This effect seems to depend, in some way unknown, upon the development of bacteria or the acids produced thereby. Incubation of a flour suspension at 27° C. in the presence of bacteria serves as a convenient means of differentiating these strongly overheated flours from normal flours.

Kent-Jones has suggested the method of incubation in auramine as suitable for the measurement of the proteolytic activity of flour, but the results of the present work intimate that this method does not give the enzymic activity of the flour, but it does give a very useful indication of the resistance of the proteins to the proteoclastic enzymes of the bacteria present. It will be seen later that the proteolytic activity of flour itself is of small magnitude. Little use has been found for Berliner and Rüter's method of incubating in pH 3.0 lactic acid, as bacterial growth is not completely inhibited and the "acid swelling" of the proteins is encountered.

Acidity Effects.—The importance of acidity in determining the physical properties of proteins has frequently been demonstrated. For example, Pauli (1922) and Loeb (1922) have discussed the viscosity of protein solutions in the presence of varying amounts of different acids. Sharp and Gortner (1923) investigated the effect of the addition of various amounts of normal acids on H-ion concentration and viscosity of flour suspensions. They obtained curves similar to those of Loeb with a maximum at about pH 3.0.

They state that at this pH the greatest imbibitional effect is produced, regardless of the nature of the acid.

It was thought to be of interest to investigate this point with the heated and overheated flours, in view of the results previously recorded.

In the preliminary experiments, the method of Sharp and Gortner was used, namely the preparation of a water suspension and the addition successively of small quantities of the acid; using 30 per cent suspensions and syrupy lactic acid, the results were satisfactory while the acid additions were small (up to about pH 3.0). In the attempts to work with greater acidity the additions were so large that a dilution effect was apparent. Sharp and Gortner were satisfied that there was no dilution error in their determinations, but it is to be noted that they did not go, in the case of lactic acid, beyond pH 3.0. In view of the abnormalities already noticed, it was necessary in the present case to go beyond that point as far as practically possible. A more satisfactory procedure was to make each suspension individually. Thus a series of acid solutions was prepared, e.g. approximately, N/400, N/200, N/80, N/40, N/20, N/4, N/2, N/1, 3N/1, 4.5N/1 in the case of lactic acid. Twenty grams of the flour was weighed out and made into a suspension with 100 cc. of the first of these solutions; it was allowed to stand for fifteen minutes and then its relative viscosity and pH were determined. A fresh 20 g. of flour was then used with the next acid solution and the observations repeated and so on through the whole series of acid solutions. The series was varied with the stronger acids so as to cover approximately the same pH range. The method was tedious, but it ensured each suspension having nearly similar treatment and obviated any dilution errors.

These viscosity and pH data were obtained with a number of acids; e.g. lactic, hydrochloric, sulfuric, nitric and acetic, and a number of interesting facts are apparent. The curves for hydrochloric, sulfuric and nitric acids are normal in that they show a peak at approximately pH 3.0; the sulfuric acid curve is considerably lower than the hydrochloric or nitric acid curves. It is of importance to observe that in two of these cases the curve for the strongly overheated flour is distinctly lower than the others; in the sulfuric acid, there is little difference.

The lactic and acetic acid curves did not show a maximum point; at a pH slightly lower than 3.0 there is a tendency to change direction, but it is not maintained and at lower pH the curve goes upward again as far as it is possible to make observations. Loeb

found a similar phenomenon with gelatin acetate when investigating the relationship of swelling to pH; his curve did not show the usual maximum, and he suggested that the high concentration of undissociated acid causes a secondary modification of the gelatin. This is quite possibly true in the case of both acetic acid and of lactic acid. The behaviour of the strongly overheated flour is striking; between pH 4.5 and 2.0 the viscosity is very much less than that of the untreated or normally heated flours, but at about pH 1.7 the curve rises very abruptly. This curve is markedly different in character from the other curves and probably is dependent on a very drastic change in the colloidal or physico-chemical properties of the heated proteins. It is of significance that this secondary effect is of very different value in the untreated and strongly heated flours.

All the data obtained are not reproduced, but Tables III and IV show the typical results in lactic and hydrochloric acids. Graphical representations are given in Figs. 3 and 4.

TABLE III

RELATIONSHIP OF VISCOSITY OF 20% FLOUR SUSPENSIONS TO THE pH OF THAT SUSPENSION IN THE PRESENCE OF LACTIC ACID

Approx:	Flour I		Flour III		Flour VI	
Acid Soln.	pH	Viscosity	pH	Viscosity	pH	Viscosity
Water	6.20	2.31	6.25	2.40	6.10	2.46
N/400	5.40	2.43	5.25	2.44	5.15	2.64
N/200	4.65	2.51	4.55	2.59	4.55	2.71
N/80	3.90	5.87	3.85	5.30	3.80	3.10
N/40	3.30	7.63	3.30	7.15	3.30	3.52
N/20	3.10	8.27	3.05	8.80	3.05	4.06
N/4	2.25	9.04	2.30	8.61	2.30	6.40
N/2	2.05	8.79	2.00	9.00	2.00	7.00
N/1	1.70	8.92	1.70	9.40	1.85	7.53
2N/1	1.55	10.44	1.55	11.36	1.55	12.60
3N/1	1.45	16.20	1.50	17.60	1.45	17.60
4.5N/1	1.33	23.00	1.35	25.00	1.30	105.00

TABLE IV

RELATIONSHIP OF VISCOSITY OF 20% FLOUR SUSPENSIONS TO THE pH OF THAT SUSPENSION IN THE PRESENCE OF HYDROCHLORIC ACID

Approx:	Flour I		Flour III		Flour VI	
Acid Soln.	pH	Viscosity	pH	Viscosity	pH	Viscosity
Water	6.20	2.31	6.25	2.40	6.10	2.46
N/1600	5.90	2.36	5.90	2.40	5.90	2.56
N/800	5.70	2.45	5.70	2.50	5.70	2.65
N/300	4.90	2.40	4.90	2.51	4.90	2.81
N/160	4.15	3.33	4.15	3.30	4.15	3.00
N/110	3.60	6.00	3.60	5.45	3.60	3.08
N/80	3.05	8.20	3.05	7.22	3.05	3.75
N/55	2.45	6.82	2.45	7.29	2.45	4.06
N/40	2.05	5.60	2.05	5.68	2.05	3.78
N/20	1.55	3.05	1.60	3.20	1.60	3.38
N/5	0.80	2.51	0.80	2.45	0.80	2.85

It is shown, therefore, that severe heat-treatment of flour produces an important physical or physico-chemical change in the proteins, which is markedly manifested in the nature of the viscosity-acidity curves.

Dough Acidity Changes During Fermentation.—Much work has been done on the H-ion concentration of flours since Jessen-Hansen (1911) published his important contribution to this subject. Generally speaking, opinion is divided as to the importance

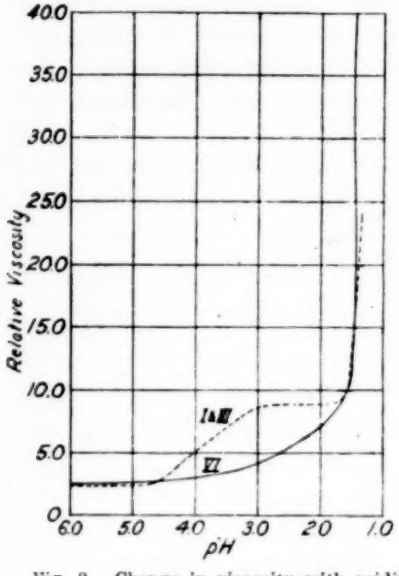

Fig. 3. Change in viscosity with acidity. Lactic acid curves. Flours I, III and VI.

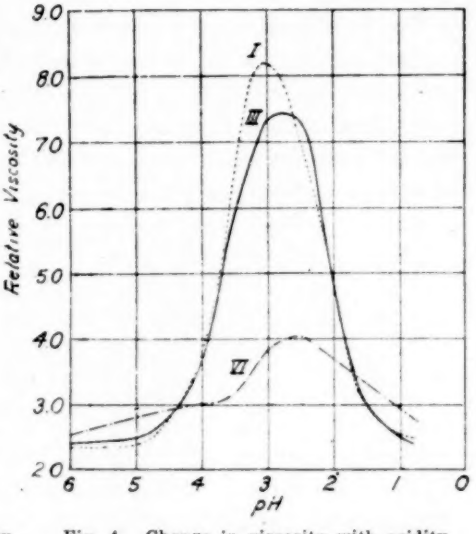

Fig. 4. Change in viscosity with acidity. Hydrochloric acid curves. Flours I, III and VI.

of acidity in fermentation. In addition to Jessen-Hansen, Cohn and Henderson (1918) and Dunlap (1922) contend that the pH of flour and dough is a determining factor in bread quality, whereas Bailey (1925) is of the opinion that any effect of increased acidity is due to the acceleration of the enzymes.

Recently the subject has been re-investigated by Fisher and Halton (1929a), who have criticized the work of Jessen-Hansen in that the margin of error in his technique was large and that he only used one system of fermentation, instead of varying his length of fermentation to suit each individual flour. Fisher and Halton followed the pH changes in doughs throughout fermentation and they found only a small change in the pH, even though the fermentation was carried to the limit when the loaf was ruined.

The author has confirmed that the pH changes of the dough during fermentation are of small magnitude; such changes during a four hours system being in the region of 0.1 to 0.2 pH. Properly fermented doughs in the author's laboratory do not reach pH 5.0 at any time, a typical figure for a dough entering the oven being 5.85 pH. It is seen from Fig. 3, therefore, that changes in pH of the dough can have no significant effects on the protein properties during the process of bread making.

It should be noted that these determinations were made using the quinhydrone method and platinum electrodes. Halton & Fisher (1928) found a drift of the pH to the acid side if platinum electrodes were used; the author confirmed this drift in many cases but not consistently and in any case the value of the drift obtained in two hours was only 0.1 to 0.2 pH, so that any error during the making of an observation (about two minutes) cannot affect the results appreciably.

Protein Solubility.—The various protein fractions generally recognized in flour were estimated in the samples under investigation in this study, as well as in many other commercial flours. These are:

(a) Proteins soluble in 5% potassium sulphate solution.
(b) Alcohol soluble proteins, usually called "Gliadin."
(c) Glutenin.

The first two were determined by the successive method of Sharp and Gortner (1923), bearing in mind the observations made thereon by Kent-Jones (1926). The glutenins were estimated by a number of methods, viz., those of Blish and Sandstedt (1925a), Blish, Abott and Platenius (1927) and Grewe and Bailey (1927). Results are given in Tables V and VI.

In the first place it must be noted that the sum of the three fractions does not equal the total protein determined directly on the flour; the sum is usually lower than the total protein in good grade flours but vice versa in lower grade flours. The agreement between various glutenin methods is fairly satisfactory. In all cases it is found that severe heating causes a distinct diminution in the solubilities of the proteins, again indicating a definite physical change having taken place.

Gortner, Hoffman and Sinclair (1929) in a recent paper have studied the solubility effects of various salt solutions. They have shown very clearly that different amounts of protein are extracted by different solutions, that is, that the salts have varying peptizing

powers on the flour proteins. It seemed to the author that if a salt be used with a higher peptizing power for the preliminary extraction then the subsequent alcohol extraction would be lower and the quantitative distinction between non-gluten proteins and gluten proteins would vanish.

TABLE V

PROTEIN DISTRIBUTION ON A SERIES OF FLOURS, THE GLUTENIN BEING DETERMINED BY THE METHOD OF BLISH & SANDSTEDT (CALCULATED TO 15% MOISTURE BASIS)

Flour	5% K_2SO_4 Soluble %	Alcohol Soluble "Gliadin" %	Glutenin %	Sum. %	Total N x 5.7 %	Difference %
I.	1.22	4.11	3.82	9.15	10.00	−0.85
II.	1.20	4.10	3.81	9.11	9.96	−0.85
III.	1.22	4.10	3.88	9.20	9.96	−0.76
IV.	1.20	4.15	3.90	9.25	9.90	−0.65
V.	1.25	4.08	3.80	9.05	9.96	−0.91
VI.	0.68	2.32	2.61	5.61	9.90	−4.29
Australian	1.52	4.59	4.62	10.73	11.05	−0.32
No. 6 Manitoba	1.65	5.16	4.68	11.49	11.06	0.43
English Patents	1.31	4.31	3.23	8.85	9.16	−0.31
"A" Machine	1.28	4.53	4.56	10.37	10.49	−0.12
"J" Machine	2.19	4.85	5.50	12.54	11.93	0.61
IVth Break	2.60	6.35	7.30	16.25	15.96	0.29
A. 277	1.20	4.42	4.21	9.83	10.29	−0.46
A. 278	1.57	4.79	4.77	11.13	10.60	0.53
C. 474	1.42	3.80	4.03	9.25	9.59	−0.34
C. 475	1.60	3.92	4.42	9.94	10.08	−0.14

TABLE VI

GLUTENIN ON VARIOUS FLOURS BY DIFFERENT METHODS OF DIRECT ESTIMATION (CALCULATED TO 15% MOISTURE BASIS)

Flour	Blish & Sandstedt	Blish, Abbott & Platenius	Grewe and Bailey I	Grewe and Bailey II	Range
I.	3.82	3.97	4.09	3.99	0.27
II.	3.81	3.80	4.40	4.26	0.60
III.	3.88	3.79	4.12	4.16	0.37
IV.	3.90	3.80	4.13	4.13	0.33
V.	3.80	3.80	4.40	4.02	0.60
VI.	2.61	6.63	6.95	6.91	...
No. 6 Manitoba	4.68	5.07	4.45	4.53	0.62
Eng. Patents	3.23	3.45	3.09	3.24	0.36
A. 277	4.21	3.87	4.04	...	0.34
A. 278	4.77	4.34	4.50	4.59	0.43
C. 474	4.03	4.14	3.58	3.47	0.67
C. 475	4.42	4.03	3.82	3.78	0.64

To test this point two flours, namely Manitoba and English, were extracted by different salt solutions, each followed in the usual way by a 70% alcohol extraction. These figures, for potassium sulphate, calcium chloride and potassium iodide solutions, together with the total salt and alcohol extracts are given in Table VII.

TABLE VII

THE SOLUBILITY OF FLOUR PROTEINS IN DIFFERENT SALT SOLUTIONS AND THE EFFECT ON THE SUBSEQUENT ALCOHOLIC EXTRACTION

Flour	5% K_2SO_4 Extract	Alcoholic Extract after K_2SO_4	Total	5% $CaCl_2$ Extract	Alcoholic Extract after $CaCl_2$	Total	5% KI Extract	Alcoholic Extract after KI	Total
	%	%	%	%	%	%	%	%	%
Manitoba	1.65	5.16	6.81	3.20	3.37	6.57	4.24	2.75	6.99
English	1.30	4.58	5.88	3.07	3.21	6.28	3.60	2.77	6.37

It is seen from Table VII that the more protein the salt solution extracts, the less the subsequent alcoholic solution will extract. The total of protein soluble in salt solution plus protein subsequently soluble in 70 per cent alcohol is approximately constant for each of the flours studied. This confirms the very important aspect of Gortner, Hoffman and Sinclair's work that the and different salts have varying powers of peptizing flour proteins. The "solutions" of flour proteins are therefore, not true solutions of chemical entities, but colloidal sols, whose composition will vary according to external influences. The overlapping of solubilities between the potassium sulphate fraction and the gliadin fraction is carried a stage further, in that the amount of "gliadin" extractable will depend on the previous treatment of the flour proteins. The chemical individuality of what is normally termed gliadin is, therefore, challenged. Gortner, Hoffman and Sinclair contend that different flours vary in the ease with which their proteins are peptized; this is apparently so with some of the proteins, e.g. those soluble in alcohol, but it is not necessarily so with the glutenin. It has been seen that the various methods for the estimation of glutenin agree moderately well, even using different dispersing agents [NaOH and $Ba(OH)_2$], and, while it may be so, it has not yet been shown that the fraction known usually as "glutenin" is as ill-defined chemically as is "gliadin."

Kent-Jones (1926) found in the flours examined by him that the stronger the flour the lower the proportion of potassium sul-

phate-soluble protein to the total protein and as Gortner, Hoffman and Sinclair (1929) intimate that the proteins of different flours are peptized to different degrees by a salt solution, it is probable that this peptizing effect depends on the physical or colloidal conditions of these proteins, which would account for Kent-Jones's observation on his own theory that the proteins of stronger flours are more coagulated, or in a state of greater aggregation, than are those of weaker flours. Heat treatment will alter the state of aggregation of the proteins and thus increase the resistance to any peptizing action. The more severe the heat treatment, the greater the increase in resistance, resulting in an apparently decreased solubility.

The use of ultra-violet radiation in detecting heat-treated flours.—The first use to be made of the short ultra-violet radiations and their effect on heat treated flour, as far as the author is aware, was by Berliner and Rüter (1928a), who observed that flour, after strong heating, shone up light blue when placed between other untreated or only lightly treated flours. Kent-Jones (1928) suggested that this peculiar luminescence might be due to the granular nature of the heated flour rather than to a specific change in its properties. Tausz and Rumm (1928) also made a number of interesting observations on the appearance of flours and wheats under the ultra-violet light, and later Berliner and Rüter (1929) extended their investigations on this subject and found that generally the luminescence of flours depended directly on the degree of coagulation of the albumin of the flour.

The author examined a large number of mill stocks under the ultra-violet radiations, including the six flours chosen for these investigations. It was found that there are two factors present:

(a) the intensity of the fluorescence, that is, the brightness of the fluorescent light.
(b) the blueness, or colour of that fluorescence.

It was apparent that the first of these increases with increasing granularity of the stock, being most prominent in the coarse semolina. The strongly overheated flours show a greater brightness than the untreated flour, being about similar to the middlings stock from this mill, but at the same time the color of the fluorescence is of a decidedly lighter blue, which difference is not seen so much in the granular but unheated stocks. This difference in colour is presumably the one observed by Berliner and Rüter and the brightness or glistening effect is the one referred to by Kent-

Jones. The decrease in color may be associated with the coagulation of the proteins, because, although the coarse semolina stock appeared to be slightly less blue than the straight run, this was not nearly so marked as with the strongly heated flour.

The difference in the appearance of the untreated and strongly heated samples did not seem to be sufficient to warrant the expectation of detecting an admixture, and this proved to be the case, even when the overheated flour was mixed in a proportion larger than those used commercially. Neither was the difference between untreated and correctly heated flours sufficient for the detection of the heat treatment. Ultra-violet radiations are, therefore, not sensitive enough to detect the normal heat treatment of commercial flours. Neither could other cereal or seed contamination be detected.

The importance of moisture on the effects of heat on the proteins in flour.—Kent-Jones in his original thesis pointed out that the effects of heating either wheat or flour were dependent on the moisture content of that stock; the greater the moisture content, the greater the changes in properties effected by a definite heating process. Berliner and Rüter in their criticism of the original work again emphasize the great importance of the moisture and report an experiment in which they extracted the moisture from a flour down to four per cent in an evacuated desiccator over calcium chloride as a drying material at a mercury pressure of 110 mms. The gluten was completely unaltered. When this flour was heated in an open vessel in the drying oven at 100° C, it bore a one hour's heating without any damage to the gluten. Another flour which had previously been dried in a vacuum at room temperature bore a fifteen hours' heating in an open vessel in the drying oven at 80° C. without any damage to the gluten. They maintain that under these conditions even a washed-out gluten does not become irreversibly coagulated in one hour at 100° C.

An investigation was, therefore, commenced on the effects of heat upon a previously dried flour. A quantity of the original flour I was placed in open trays in an incubator, maintained at a temperature of 30° C., with provision for the escape of moisture vapour. It was kept thus until the moisture content was in the region of 4.5 per cent when it was removed and stored in stoppered bottles.

This dried flour was then used as the basis for the preparation of heat treated flours, in the same way as the original flours II to VI had been prepared; a preliminary test indicated that after

eleven hours at 180° F., the gluten could still be washed out, so that the time of heating was lengthened. Samples of the dried flour were heated for 11, 22, 46 and 112 hrs. at 180° F. Gluten could be washed out of all, except that which had been heated for 112 hours. The flours thus obtained were examined with the object of detecting critical changes which had taken place. From the present work, it appears that the general criteria of changes effected by heat may be taken as:

(a) alteration in viscosity-acidity curves, e.g., lactic acid is typical.
(b) reduction of solubility of proteins.

The viscosity-acidity curves in lactic acid were obtained for these flours; they are not reproduced, but it was found that only after 112 hours' heating did the curve show any of the typical characteristics of that of the overheated flour.

It was found that even after the 112 hours' heating the viscosity curve had not assumed completely the nature of that of flour No. VI. There was undoubtedly a tendency that way, but the ascending portion of the curve was less steep than that of flour No. VI. If this can be taken as an indication of the change to the proteins, then it would appear that 112 hours' of heating the flour in a more or less dry state has modified the properties, but that the change is not as great as in ten hours' heating in the presence of the natural moisture.

The protein solubility in 70% ethyl alcohol at 20° C. was determined on these flours and the figures are given in Table VIII, the protein percentages being calculated onto the dry flour basis.

TABLE VIII

PERCENTAGES OF PROTEIN SOLUBLE IN 70% ALCOHOL FROM FLOURS HEATED FOR VARIOUS TIMES IN THE PRESENCE OF ONLY SMALL AMOUNTS OF MOISTURE (CALCULATED TO DRY BASIS)

Time of Heating	% Protein Soluble in 70 Per Cent Alcohol
0 hours	4.68
11 hours at 180° F.	4.71
22 hours at 180° F.	4.70
46 hours at 180° F.	4.65
112 hours at 180° F.	4.59

Heating in the absence of moisture causes a much smaller effect on the solubility of proteins than a corresponding treatment in the presence of the natural moisture content of the flour. There is, however, evidence that after 112 hours at 180° F. the solubility

of the proteins in 70% ethyl alcohol is slightly reduced, but that this effect is much smaller than observed after heating normal flour for 10 hours at 180° F. This suggests, in agreement with the viscosity observations, that the effects of heat on the proteins of this 112 hours flour have not been so marked as in the case of flour No. VI in the earlier part of this paper.

The effectiveness of these flours as "improvers" increased as the heating was continued, but, finally was not quite so good as original flour No. VI. The parallelism between "improving" action and the protein changes naturally suggests that the two phenomena are connected.

In the presence of this small amount of moisture, the author finds in opposition to Berliner and Rüter that the proteins of flour can be coagulated and the same effects produced but that the speed of this physical change is very much diminished.

Discussion

A study has been made on the effects of heat on the proteins of wheaten flour. The heating of flour to improve its baking quality is now being used commercially in many English and European mills with very satisfactory results. The "improving" effect can be accomplished in one of two ways:

(i) a relatively large proportion of the flour is heated, or
(ii) a very small percentage is heated to a severe degree and then mixed back at the rate of 0.7 to 1.0%.

According to the results of this investigation the heating of the whole of the flour to give it a maximum baking quality causes a small but definite change in the imbibitional powers of the proteins, without any startling changes in solubilities or physical properties.

The severe heating required to produce the "improver" mentioned in (ii) above causes very marked changes in imbibitional and physical properties of the proteins. Incubation experiments in the presence of bacterial development show an enormous increase in the viscosity of the overheated flour suspension, such an increase being greater than that for any acid studied at a corresponding pH. If the growth of bacteria is inhibited, then this remarkable increase in viscosity does not occur. Bacteria or the acids produced by them, do, therefore, play an important part in the viscosity changes of a flour suspension during incubation. The possibility of any role

during the comparatively short fermentation period must be left to bacteriologists for an answer.

These protein changes are accelerated by the presence of moisture in the flour; flour has not been obtained absolutely dry, so it cannot be stated what would be the effect of heat in the complete absence of moisture. At about 4% water content, however, the same changes in the proteins can be produced so long as the time of heating is sufficiently prolonged.

The changes in the proteins are of fundamental importance and it is on these that the improvement in strength of the flour is based. This modification of the proteins of a flour, heated to give maximum baking strength, increases the imbibitional power and thus has a direct effect on the gluten fibrils of the dough structure. In addition, it is suggested that the resistance to proteolytic attack is somewhat increased. This change is probably due to an increase in degree of the aggregation of the protein particles. The exact phenomenon is not clear; Kent-Jones believes that the two stages of denaturation and flocculation occur in most samples, but that in some cases the protein is denatured by heat and the flocculation is only brought about by the acids produced during fermentation. Lewis (1926) has affirmed that the water of hydration of proteins plays a definite role in their denaturation, and Jordan Lloyd (1926) states:

> "The change involved in denaturation seems to be a structural alteration in the protein molecule, but not an actual degradation. This change is accompanied by a complete loss of the power of swelling by imbibition of water."

It is doubtful, therefore, if the changes involved in flour heating are strictly a denaturation, but all the evidence does lend itself to a theory of physical alteration, involving an increase in the imbibitional power. The process is a gradual one and can be carried to various stages, according to the conditions and severity of the heating. The amount of moisture in the flour, is, probably, the main factor determining the extent of this protein modification, under a given time and temperature.

Summary

Kent-Jones' viscosity curves in 1:10,000 auramine are confirmed, but it is shown that bacterial development and increase in acidity take place, and, therefore, no measurement is afforded of the proteolytic activity of the flour itself. Viscosity changes, how-

ever, may give a measurement of the resistance of the proteins to proteolytic attack.

Incubation in pH 3.0 lactic acid did not completely inhibit bacterial growth; the viscosity curves are very different from those in water.

Incubation in the presence of one per cent ammonium fluoride solution gave apparently no bacterial growth; the viscosity *decrease* in the untreated flour was less and the viscosity *increase* in the strongly heated flour was also less.

Viscosity-acidity curves show interesting differences in the unheated and the strongly overheated flours, giving evidence of marked physical changes in the flour proteins.

Many of the well known bactericidal substances were found to be insufficient for inhibiting the growth of bacteria in flour-water suspensions.

Various methods for the estimation of glutenin gave fairly good agreement between one another.

The sum of the three fractions of proteins by recognized methods of analysis did not agree in many cases with the total protein figure determined directly on the flour; in good grade flours the sum is usually lower than the total, whereas in the lower grade flours the sum is frequently higher than the total.

Different salts have different peptizing effects on some of the flour proteins; a subsequent alcohol extraction will vary according to the preliminary salt treatment, indicating that the so-called "gliadin" is not a characteristic entity.

The solubility of all proteins in their respective solvents is diminished by severe heating, indicating increased resistance to peptization.

Ultra-violet radiations do not apparently fulfil any useful purpose in cereal analysis.

If a large proportion of the natural moisture of a flour be removed at a low temperature and then the heating performed, the effects on the flour proteins will be produced but at a very much slower rate.

Acknowledgment

The author desires to place on record his great appreciation of the continual interest which Dr. D. W. Kent-Jones has taken in these researches.

Since this thesis was presented, the works relevant to this subject matter of Geddes and Blish and Sandstedt have been received by the author.

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THE STABILITY OF LEAVENING IN SELF-RISING FLOUR

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(Read at the Convention, May, 1930)

Self-rising flour now constitutes approximately 10% of the country's flour consumption (See Fig. 1). According to LeClerc and Bailey (1929) it amounts to 21.7% of the flour consumed by the housewife. The production of self-rising flour, practically all of which is consumed in the United States, has increased for the past five years at a cumulative rate of 3.29% annually. (See Fig. 2.) Contrasted with this the total flour consumption during the same period has increased at the rate of 1.55% annually, a value lower than the rate of population increase of 1.65%. In other words, whereas the total per capita flour consumption of the United States is declining, the use of self-rising flour is increasing at a rate double that of the population increase of the country.

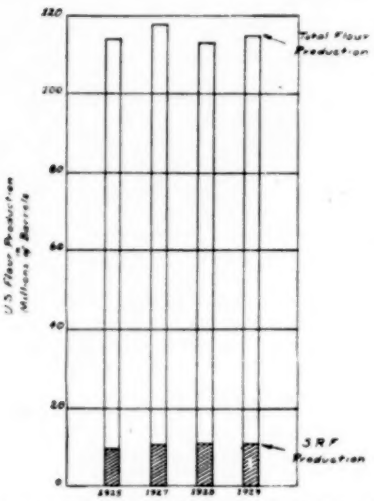

Fig. 1. Relation of Self-Rising Flour to Total Production in the U. S.
Data of LeClerc & Bailey and Pope.

The 1927 Census of Manufacturers gives the baking powder produced in the United States as 155,941,240 lbs. In the same year the leavening ingredients in self-rising flour calculated to a 14% baking powder would have amounted to 107,500,000 lbs. or 68.9% of the quantity manufactured as baking powder straight. A survey of these figures indicates that self-rising flour is of considerable economic importance to the nation and of more than passing interest to flour millers and cereal chemists.

The question is raised, however, whether the leavening ingredients in self-rising flour actually retain their gas-evolving power through the normal period elapsing between the production and consumption of the flour. This question is based on the fact that flour normally contains more moisture than the starch commonly used as a filler in baking powder and that self-rising flour is not usually packed in moisture-resisting packages such as

cans used for baking powder. A self-rising flour having 10% moisture, for example, will contain 7.03 lbs. water per pound of soda, which would at first glance be enough to cause complete decomposition of all leavening agents contained. On the other hand a 14% (i.e., 14% CO_2) phosphate baking powder filled with starch containing 7% moisture has only 0.104 lbs. water per pound of soda. The case against self-rising flour is not so unfavorable, however, as a comparison of these figures would indicate. Account must be taken of the relative concentrations of the reacting constituents in each mixture and the magnitude of the attractive force with which the water is held.

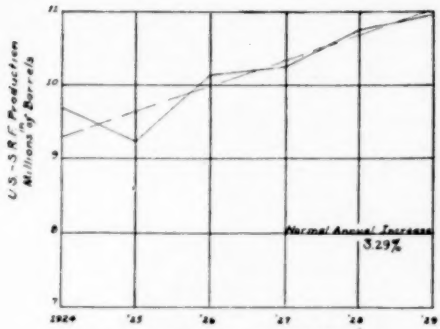

Fig. 2. Trend of U. S. Self-Rising Flour Production.

Data of F. T. Pope.

Experimental

To determine the stability of leavening in self-rising flour, flours were selected having moisture contents of 11.32%, 12.43%, 13.22% and 14.02% respectively, and were made into self-rising flour according to the usual formula: 100.00 g. flour, 1.50 g. soda, 1.75 g. phosphate, and 2.00 g. salt. The samples in duplicate were stored in tin cans having the cover-joints sealed with adhesive type to maintain the moisture content as nearly uniform as possible. The samples were tested periodically over a year with the result shown in Table I.

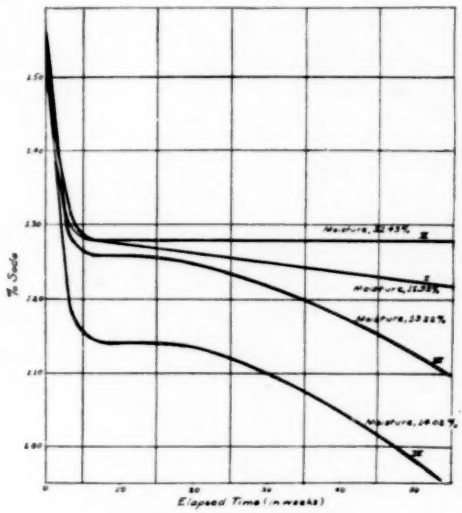

Fig. 3. Soda Deterioration in Stored S. R. F. of Varying Moisture Content.

Discussion

It will be seen that the available soda (Fig. 3) as measured

TABLE I

EFFECT OF DIFFERENT MOISTURE CONTENTS IN STORED SELF-RISING FLOUR ON DETERIORATION OF LEAVENING AND ON pH, LIGHTNESS AND VOLUME

Time Weeks	Soda %	pH	Lightness[1]	Volume[2]
		A. Flour Moisture 11.32%		
0	1.55	7.1	14.40	29.00
1	1.43	7.05	15.05	29.70
2	1.37	7.05	14.43	29.30
3	1.30	7.1	14.72	29.85
5	1.28	7.1	15.10	30.15
7	1.30	7.05	15.50	31.00
11	1.32	7.1	15.30	30.68
20	1.28	7.1	15.25	30.45
34	1.28	7.0	14.50	29.45
53	1.22	7.0	14.23	28.65
		B. Flour Moisture 12.43%		
0	1.55	7.1	14.50	29.48
1	1.37	7.1	14.50	29.05
2	1.34	7.05	15.17	30.15
3	1.28	7.1	14.60	29.70
5	1.28	7.1	14.84	29.70
7	1.28	7.05	15.90	31.68
11	1.28	7.05	15.74	31.28
20	1.28	7.05	15.30	30.90
34	1.30	7.05	15.00	29.85
53	1.34	7.1	14.10	28.40
		C. Flour Moisture 13.22%		
0	1.56	7.1	14.78	30.00
1	1.41	7.2	15.40	30.40
2	1.39	7.15	15.00	30.15
3	1.26	7.2	14.53	29.58
5	1.26	7.25	14.60	30.15
7	1.28	7.25	15.12	30.70
11	1.26	7.3	15.74	31.40
20	1.26	7.35	15.72	30.55
34	1.20	7.5	13.95	27.95
53	1.11	7.5	13.28	26.60
		D. Flour Moisture 14.02%		
0	1.51	7.1	15.09	30.55
1	1.39	7.1	15.40	30.28
2	1.30	7.15	14.78	29.70
3	1.20	7.2	14.94	30.00
5	1.20	7.3	14.22	29.58
7	1.16	7.3	15.38	31.00
11	1.14	7.35	14.90	30.00
20	1.14	7.4	15.46	30.15
34	1.07	7.6	14.62	29.20
53	0.96	7.6	13.17	26.30

[1] Lightness = $\frac{\text{Specific volume} \times 15}{\text{Specific vol. of Standard}}$

[2] Volume = $\frac{\text{Biscuit vol. in cc./grams of dough} \times 30}{\text{Vol. of standard biscuit in cc./grams of dough}}$

by the gravimetric determination of CO_2 evolved, decreased rapidly at first and then more slowly as time elapsed. This did not

seem to have a commensurate influence on the lightness and volume (Fig. 4) of the biscuit produced, however, since these showed in every case a tendency to improve at some portion of the storage period. The explanation of this is believed to lie in the fact that these flours were quite fresh at the beginning of the test and ageing improved the baking quality of the flour itself, thus more than offsetting the effect of the loss of leavening strength.

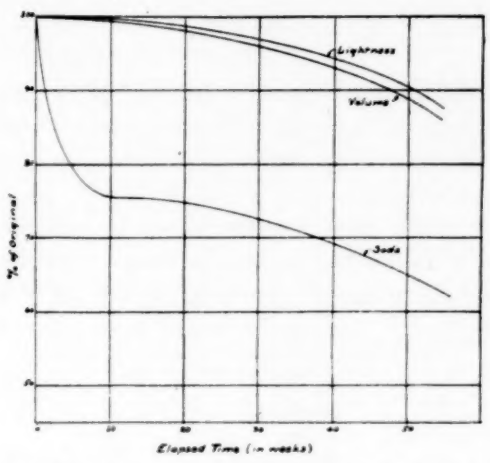

Fig. 4. Changes in Lightness, Volume and Soda Content of S. R. F. in Storage.

The rise in pH, noted especially in the case of flours with higher moisture content is believed to be due to the character of the reaction which takes place under the conditions of premature action in self-rising flour (Fig. 5). Mono-Calcium Phosphate and sodium bicarbonate react in accordance with one or more of the following reactions:

TABLE II

THE REACTION OF MONO-CALCIUM PHOSPHATE AND SODIUM BICARBONATE

A. $CaH_4P_2O_8 + 2NaHCO_3 = CaHPO_4 + Na_2HPO_4 + CO_2 + 2H_2O$
B. $3CaH_4P_2O_8 + 4NaHCO_3 = Ca_3P_2O_8 + 4NaH_2PO_4 + 4CO_2 + 4H_2O$
C. $3CaH_4P_2O_8 + 8NaHCO_3 = Ca_3P_2O_8 + 4Na_2HPO_4 + 8CO_2 + 8H_2O$

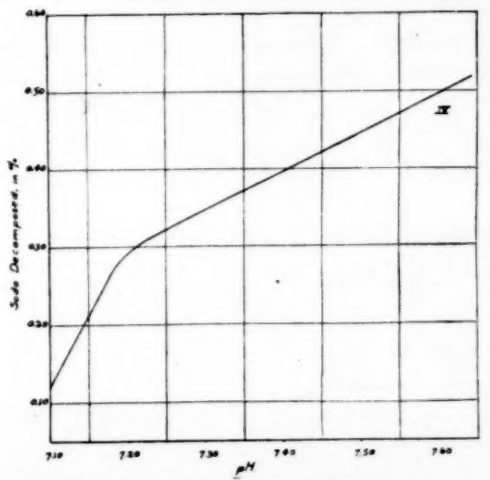

Fig. 5. Effect of Premature Reaction of Soda on pH of Biscuit.

The reactions indicated by these equations are subject to the variables: temperature, concentration, and relative proportions of reactants. Low temperature favors A; excess alkali favors C. High temperature favors B and C.

From the above equations it is seen that one mol $CaH_4P_2O_8$ reacts with the following mols of $NaHCO_3$:

TABLE III

MOLS OF SODIUM BICARBONATE REACTING WITH ONE MOL OF MONO-CALCIUM PHOSPHATE

Equation	Mols. $NaHCO_3$ per mol. $CaH_4P_2O_8$
A	2.00
B	1.33
C	2.67

When mono-calcium phosphate is titrated with NaOH in the cold, a value is obtained which indicates that 100 parts commercial mono-calcium phosphate will neutralize about 72 parts sodium bicarbonate. Calculating the $CaH_4P_2O_8$ content of M. C. P. from the soluble P_2O_5 (52.2/.607) we find that commercial mono-calcium phosphate is about 86% $CaH_4P_2O_8$. On the above basis, then, 86 parts $CaH_4P_2O_8$ neutralized 72 parts sodium bicarbonate. From these figures we may calculate:

$$\frac{72\ NaHCO_3 \times 234\ CaH_4P_2O_8}{86\ CaH_4P_2O_8 \times 84\ NaHCO_3} = 2.33\ \text{mols}\ NaHCO_3$$

per mol $CaH_4P_2O_8$ in the cold. This value indicates that equation A and/or B are involved together with C in this reaction. If, on the other hand, the reaction mass is heated to boiling, more alkali may be added. The phosphate then shows a neutralizing value of around 78 and by a calculation similar to that above we learn that an equivalent of 2.53 mols $NaHCO_3$ per mol $CaH_4P_2O_8$ is used. Boiling apparently increases the reaction according to equation C at the expense of the earlier reactions according to A or B. If alkali is added slowly in titrating mono calcium phosphate not as much will be taken up as when it is added rapidly. This must be analogous to what happens in the premature decomposition of leavening in self-rising flour. The phosphate is used up before all the soda has reacted leaving a residue of sodium bicarbonate to be decomposed in the oven to the sesqui-carbonate which renders the final product alkaline.

Conclusion

1. The consumption of self-rising flour in the United States is increasing more rapidly than the consumption of plain flour.
2. The rate of premature decomposition of self-rising flour varies directly as the moisture content.
3. Self-rising flours containing less than 12.5% moisture are relatively stable.

4. Self-rising flours containing more than 12.5% moisture decompose more rapidly and result in alkaline baked products, unless this premature decomposition is compensated for in increased acid leavening agent.
5. An explanation of the increased alkalinity accompanying premature decomposition is offered in the mechanism of the reactions involved.

Acknowledgment

Grateful acknowledgment is made to Mr. V. E. Fisher of the Stanard-Tilton Milling Co., Alton, Ill., for the samples of flour of varying moisture contents used in the experimental work.

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THE MARCH OF ACIDITY IN STORED FLOUR. II[1]

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(Received for publication May 15, 1930)

In the first section of this study, reported by Fifield and Bailey (1929), particular attention was given to the relation between flour grade, temperature, and the rate of change in the acidity of flour as determined by the Greek and the tentative A. O. A. C. methods. This demonstrated that the rate of change in acidity was a function of flour grade and of temperature, particularly in the early period of storage.

It then appeared desirable to check these earlier experiments, which involved only six samples of flour, through the use of a larger number of flours, in order to ascertain the relative consistency of the rate of change of acidity among flours of the same relative grade or degree of extraction. To this end the cooperation of the Committee of Flour Specifications of the American Asso-

[1]Published with the approval of the Director as Paper No. 976, Journal Series, Minnesota Agricultural Experiment Station.

ciation of Cereal Chemists was solicited, and members of the committee—Miss Sullivan, Mr. Flohil, Mr. Olsen and Dr. Collatz—provided a total of 52 samples for this purpose. Among these were included unbleached straights, first clears, and second clears milled from hard red spring wheat, and unbleached first clears milled from durum wheats.

The flours were sent to the laboratory directly from the mill where they were produced, in order that a minimum of time might elapse before the acidity of the freshly milled flour was determined. All these initial analyses were completed within a period of four days from the time the flours were produced in the mill, and in the majority of instances within two days or less. These chemical determinations are later referred to as "analyses of freshly-milled flour."

A portion of each sample was placed in a cotton sack as soon as received, and these sacks were in turn stored in air thermostats maintained at 25±.1° C. and at a relative humidity of 80±%.

Chemical determinations on the freshly milled flours were confined to moisture, ash, H-ion concentration, acidity by the Greek method, and acidity by the A. O. A. C. tentative method. After the lapse of about 80 days of storage 35 of these samples were reanalyzed as before, except that the ash content was not redetermined. The resulting data are recorded in Table I on the basis of the moisture content at the time of sampling. In these tables the acidity as determined by the A. O. A. C. tentative method, calculated as lactic acid in the conventional manner, and likewise as H_2SO_4 have both been recorded. The latter value was included to afford a more direct comparison with the results obtained by the Greek method, in which the acidity is also reported as H_2SO_4.

The details of these two acidity methods were included in the first paper and need not be repeated here. Suffice it to say that in the Greek method the flour was extracted with 85% alcohol overnight and an aliquot titrated with curcumâ as an indicator. In the A. O. A. C. method the flour was extracted with water at 40°C. for one hour, and an aliquot of the extract titrated with phenolphthalein as the indicator.

Results of Analyses of Freshly Milled Flour

The three grades of unbleached hard red spring wheat flour that were studied, namely, straights, first clears, and second clears, were readily distinguished by their ash content. In the instance of the straight grades the range in the percentage of ash was from

0.42% to 0.53%; in the instance of the first clears the range was 0.64% to 0.88%; while with the second clears the range was from 1.03% to 2.09%. It is evident that there was no overlapping of ash content among the several grades. The durum first clears ranged between 1.01% and 1.80% of ash, thus resembling, in this characteristic at least, the spring wheat second clears.

H-ion concentration, determined electrometrically, was highest in the instance of the straight grade flours and lowest in the instance of the second clears. This is the usual relationship between flour grade and H-ion concentration, the lower degree of acidity in the low grade flours being a consequence of their higher buffer value.

Acidity as determined by the Greek method in the freshly milled flours increased progressively from the higher to the lower grades, but the differences in this regard were of smaller magnitude than were observed in the instance of freshly milled flour when acidity was determined by the A. O. A. C. tentative method.

The coefficient of correlation of acidity by the Greek method (g) and the ash content (a) was $r_{ga} = .60 \pm .06$, while the correlation between ash content and acidity by the A. O. A. C. tentative method (w) was $r_{wa} = .84 \pm .03$ (Table IV).

In the previous paper by Fifield and Bailey appeared the statement (p. 533) concerning the A. O. A. C. tentative method that "the determination really measures the capacity of flour to produce acids as well as the initial concentration of acids before the extraction medium is applied." This assumption receives support in the observations made upon these 52 samples of flour in a freshly milled state. Thus it will be observed by scanning the data in Table I that there is a relatively high correlation between the ash content as representing the degree of refinement of the flour (a) and the difference between the A. O. A. C. acidity (recorded as H_2SO_4) and the Greek acidity (w-g). To test this general statement the coefficient of correlation of these two indices was computed and found to be $r_{a(w-g)} = .96 \pm .01$. (Table IV.)

This difference in the results of determinations by the two acidity methods is attributed very largely to the biochemical processes which go forward during the extraction of flour with water at 40° C. While conceivably the acid-reacting substances extracted by the two solvents might be somewhat different, the probabilities are that the relative rate of formation of acid-reacting substances is at a much higher level in the lower grades than in the high grade flours; in fact these observations suggest that the difference

TABLE I

RESULTS OF INITIAL AND SECOND ANALYSIS OF FLOURS STORED AT 25° C., AND AVERAGE RATE OF CHANGE PER DAY IN ACIDITY

First Analysis

Sample Number	Moisture %	Ash %	Greek Acidity %	A.O.A.C. Acidity As H_2SO_4 %	A.O.A.C. Acidity As Lactic %	A.O.A.C. Acidity Minus Greek Acidity	H-Ion Conc. as pH
Hard Spring Wheat—Straight Grade Flour							
12230-W	10.62	0.50	0.047	0.074	0.136	0.027	6.26
12234-P	13.08	.48	.051	.076	.140	.025	5.97
12242-I	11.58	.47	.044	.071	.130	.027	6.22
12245-R	12.18	.46	.039	.072	.131	.033	6.22
12249-W	12.10	.48	.043	.058	.106	.015	6.17
12252-P	11.84	.53	.044	.057	.105	.013	6.17
12258-R	12.20	.47	.041	.067	.122	.026	6.19
12262-P	12.66	.47	.047	.056	.102	.009	6.27
12266-W	12.58	0.51	0.050	0.053	0.097	0.003	6.09
Hard Spring Wheat—First Clear Flour							
12231-W	12.36	0.77	0.054	0.114	0.209	0.060	6.31
12235-P	11.72	.74	.054	.119	.161	.065	6.37
12243-I	11.74	.77	.050	.112	.206	.062	6.42
12246-R	11.80	.65	.049	.099	182	.050	6.39
12250-W	11.98	.88	.050	.108	198	.058	6.48
12253-P	10.74	.79	.046	.107	196	.061	6.40
12256-I	11.86	.74	.052	.107	197	.055	6.44
12259-R	12.40	.66	.046	.101	.185	.055	6.42
12263-P	11.06	.77	.049	.083	152	.034	6.46
12267-W	12.64	0.85	0.049	0.101	0.186	0.052	6.39
Hard Spring Wheat—Second Clear Flour							
12232-W	12.46	1.80	0.099	0.251	0.461	0.152	6.48
12236-P	11.58	1.52	.076	.190	.349	.114	6.39
12244-I	13.16	1.23	.059	.173	.317	.114	6.49
12247-R	11.46	1.06	.047	.144	.264	.097	6.49
12248-W	12.08	2.09	.066	.220	.404	.154	6.59
12254-P	11.28	1.52	.052	.169	.310	.117	6.58
12257-I	11.44	1.13	.067	.160	.294	.093	6.51
12260-R	11.04	1.11	.049	.140	.257	.091	6.51
12264-P	10.84	1.70	.056	.172	.315	.116	6.58
12268-W	12.82	1.68	0.065	0.205	0.376	0.140	6.48
Durum Wheat—First Clear Flour							
12233-W	12.50	1.09	0.083	0.149	0.273	0.066	6.46
12237-P	11.62	1.67	.088	.242	.444	.154	6.53
12251-W	11.86	1.06	.047	.108	.198	.061	6.67
12255-P	11.08	1.54	.054	.154	.283	.100	6.59
12265-P	10.68	1.80	.054	.187	.343	.133	6.56
12269-W	13.10	1.03	0.049	0.113	0.208	0.064	6.47

RESULTS OF INITIAL AND SECOND ANALYSIS OF FLOURS STORED AT 25° C., AND AVERAGE RATE OF CHANGE PER DAY IN ACTIVITY

Second Analysis

Days Stored	Moisture %	Greek Acidity %	A.O.A.C. Acidity As H_2SO_4 %	A.O.A.C. Acidity As Lactic %	H-ion Conc. as pH	Average Change per Day: Greek Acidity ($\times 10^{-4}$)	Average Change per Day: A.O.A.C. Acidity as H_2SO_4 ($\times 10^{-4}$)
			Hard Spring Wheat—Straight Grade Flour				
82	12.76	0.082	0.091	0.167	6.09	4.39	2.09
82	13.24	.073	.087	.160	5.83	2.68	1.38
79	13.16	.067	.077	.141	6.07	3.06	0.73
79	13.04	.066	.074	.136	6.10	3.38	0.29
83	13.12	.064	.083	.152	6.20	2.55	3.02
83	13.26	.068	.089	.163	6.17	2.93	3.85
80	13.00	.056	.081	.148	6.07	1.94	1.74
78	13.62	.069	.071	.130	6.00	2.82	1.96
77	13.86	0.073	0.054	0.099	6.09	2.92	0.13
			Hard Spring Wheat—First Clear Flour				
82	13.26	0.127	0.155	0.284	5.93	8.90	5.00
82	12.60	.109	.147	.269	6.10	6.83	3.35
79	13.08	.105	.140	.257	6.20	7.20	3.55
79	12.90	.086	.119	.218	6.24	2.78	2.48
83	12.88	.130	.205	.376	6.26	9.59	11.69
83	12.68	.109	.158	.290	6.26	7.66	6.12
80	12.78	.106	.174	.319	6.19	6.74	8.32
80	12.70	.084	.154	.282	6.20	4.71	6.58
78	13.60	.110	.145	.267	6.15	8.23	8.00
77	13.62	0.115	0.141	0.260	6.19	8.57	5.21
			Hard Spring Wheat—Second Clear Flour				
82	12.76	0.217	0.341	0.626	6.07	14.39	10.97
82	12.46	.157	.250	.459	6.32	9.88	7.32
79	12.58	.173	.227	.417	6.29	14.37	6.84
79	13.04	.131	.223	.409	6.14	10.63	10.00
83	12.52	.264	.298	.547	6.15	23.83	9.40
83	12.64	.248	.368	.675	6.37	23.63	24.00
80	12.82	.188	.296	.544	6.20	16.96	17.04
80	12.68	.105	.275	.505	6.29	7.09	16.90
78	13.48	.227	.290	.532	6.31	21.95	15.19
77	13.38	0.246	0.298	0.547	6.32	23.48	12.08
			Durum Wheat—First Clear Flour				
82	12.58	0.158	0.205	0.376	6.10	9.15	6.83
82	13.54	.211	.259	.475	6.34	13.70	2.07
83	12.88	.178	.208	.382	6.34	16.93	12.10
83	12.44	.232	.344	.632	6.39	21.49	22.90
78	13.60	.188	.275	.505	6.37	17.21	8.79
77	13.87	0.170	0.190	0.348	6.31	18.25	9.75

between the results of the two acidity determinations might serve as a fair criterion of flour grade, and insofar as certain characteristics of the flour are concerned might be more useful in this connection than the ash content. The acidity as determined by the two methods was then correlated and the coefficient was found to be $r_{gw} = .62 \pm .06$. (Table IV.)

Change in Acidity During 80 Days Storage

The principal purpose of this study was to ascertain the relative rate of change of acidity as determined by the two methods during the lapse of time when numerous samples of the same relative grade were compared. At the conclusion of our investigations the data from 35 samples were available.

A storage period of 80 days was deemed well suited to this purpose because the earlier studies had shown that during this interval of time the progressive change in acidity could be plotted as an approximately straight line; in other words, the rate of change was reasonably constant. When flours were stored for more than 90 days it was found that the rate of change progressively diminished thereafter in the instance of the lower grades of flours.

The relative rate of change at 25° C. was expressed for convenience as the average change per day. Using the A. O. A. C. acidity method (stating acid concentration as H_2SO_4) the rate in the instance of the hard spring wheat straight grade flours ranged from 0.13×10^{-4} to 3.85×10^{-4}. When the Greek acidity method was employed the range within the same flour was from 1.94 to 6.74×10^{-4}. The rate of change of acidity in the instance of the hard spring wheat first clear flours ranged from 2.48 to 11.69×10^{-4}; in the second clear from 6.84 to 24.00×10^{-4}.

It is thus evident that there was some overlapping in progressing from the higher to the lower grades of flour. The significance of this overlapping is likely to be over-emphasized, however, when stated in the terms recorded above, and a much more satisfactory test of the relation between grade and rate of change in acidity is afforded by determining the coefficient of correlation of ash content with the average daily increase in acidity.

When these constants were computed for these variables, the correlation of ash content (a) with the average daily increase of acidity by the Greek method (b) was $r_{ab} = .88 \pm .03$; similar correlation between ash content and average daily increase in acidity by the A. O. A. C. method (c) was $r_{ac} = .89 \pm .02$. (Table IV.)

This indicates that the overlapping that was observed did not have a very large effect upon the relationship between ash content and the rate of change, and accordingly the relative rate of change in acidity on storage can be predicted with a fair degree of certainty from the ash content.

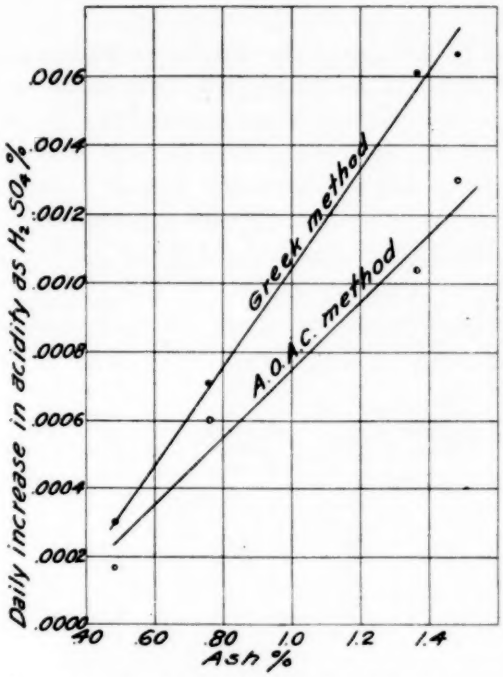

Fig. 1. Graphs showing the rate of change in acidity of flours of varying ash content, as determined by the Greek, and the A.O.A.C. methods and recorded as H_2SO_4.

TABLE II

INITIAL ACIDITY AS DETERMINED BY THE GREEK, AND A.O.A.C. ACIDITY METHODS AS H_2SO_4, AND RATE OF CHANGE IN ACIDITY THRU AN 80 DAY PERIOD, AVERAGED BY GRADES

Grade	Ash %	Acidity by Greek Method			Acidity by A.O.A.C. Method		
		Initial %	80 Days Storage	Change Per Day x 10^{-4}	Initial (as H_2SO_4) %	80 Days Storage	Change Per Day x 10^{-4}
Spring wheat Straight grade	0.48	0.045	0.069	2.96	0.065	0.078	1.69
Spring wheat First Clear	0.76	.050	.107	7.11	.105	.153	6.03
Spring wheat Second Clear	1.48	.063	.196	16.62	.182	.286	12.97
Durum wheat First Clear	1.36	0.062	0.191	16.12	0.159	0.244	10.41

A factor for computing the average daily increase in acidity (as H_2SO_4) as determined by the two methods from the ash content of the sample can be derived from the graphic record in Figure 1. These graphs are based upon the averages of the change per day, as recorded in Table II, and it will be noted that they closely approach falling upon a straight line in the instance of both acidity methods.

In the July, 1930, issue of the American Miller[2] it was reported that the official Greek Gazette recently contained new regulations governing the composition of wheat flour offered for sale in Greece. It is required that the odor of the flour shall be that of good quality flours, and its taste neither bitter nor rancid. The flour shall not be altered in any way or deteriorated, and shall be free of any adulteration made in order to conceal its alteration or to make it appear as a flour of a superior quality.

The limits of certain constituents in each quality of flour are to be as follows:

Qualities of Flour	Water Maximum	Gluten Minimum	Sulphuric Acidity Maximum	Ash Maximum	Bran Maximum	Fatty Substances Maximum
	%	%	%	%	%	%
First	13	26	0.07	0.6	0	1.10
Second	13	28	0.15	1.4	2	2.75
Standard	13	28	0.10	1.0	1	1.40
Clears	13.5	26	0.15	1.6	13	1.70
Semolina	13	28	0.07	0.8	0	1.10

"The fatty substances present in the flour are taken only as an auxiliary factor whenever there exist any doubts as to the quality. In addition to the above requirements, the following provisions of the Greek regulations are also to be taken into consideration: (a) an increase of 5 per cent in the percentage of water will be tolerated from September 15 to June 15 of every year; (b) the natural properties of the gluten shall be those of gluten contained in a good quality flour; (c) a tolerance of 10 per cent will be permitted in the acidity of the flour from June 15 to September 15 of every year; (d) in case the percentage of ash should exceed the maximum limit shown in the above table, the importation or consumption of the flour will be permitted only by decision of the council of chemical services."

[2]Grecian regulations govern flour quality. The American Miller 58: 640.

Using the data appearing in Table II, under the column "initial %" in the instance of acidity by the Greek method, and interpolating between the average values that are recorded, it appears that freshly milled first quality flour containing 0.6% ash can be expected to have an acidity by the Greek method of 0.047%; freshly milled Standard flour containing 1.0% ash should have an initial acidity of 0.055%; second quality flour containing 1.4% ash should have an initial acidity of 0.062%, and clears containing 1.6% ash an initial acidity of 0.066%. These estimated percentages of initial acidities of such flours are shown in Table III.

TABLE III

ESTIMATED TIME REQUIRED IN STORAGE AT 25° C. TO REACH MAXIMUM ACIDITY PERMITTED UNDER RECENT GREEK REGULATIONS IN INSTANCES OF FLOURS CONTAINING THE PERCENTAGE OF ASH ALLOWED IN EACH OF THE FOUR QUALITIES OF FLOUR

Quality of flour	Ash content permitted	Estimated initial acidity by Greek method	Average change in acidity per day	Time required in storage at 25° C. to reach maximum permitted
	%	%	%	days
First	0.6	0.047	0.00046	50
Second	1.4	0.062	0.00165	53
Standard	1.0	0.055	0.00105	43
Clears	1.6	0.066	0.00195	43

TABLE IV

COEFFICIENTS OF CORRELATION OF SEVERAL VARIABLES INCLUDED IN THE ACIDITY STUDY

	First Analysis	After Storage Period
r_{ag}	$0.60 \pm .06$	$0.88 \pm .03$
r_{aw}	$.84 \pm .03$	$.92 \pm .02$
r_{ap}	$.76 \pm .04$	$.64 \pm .07$
r_{gw}	$.62 \pm .06$	$.92 \pm .02$
r_{gp}	$.38 \pm .08$	$.48 \pm .09$
r_{wp}	$.63 \pm .06$	$.55 \pm .08$
$r_{a(w-g)}$	$0.96 \pm .01$	
r_{ab}		$.88 \pm .03$
r_{ac}		$0.89 \pm .02$

CODE

Ash % . . . . . = a
Acidity of fresh flour, Greek Method . . . . = g
Acidity of fresh flour, A.O.A.C. Method . . . = w
pH of fresh flour . . . . . = p
Average daily increase in acidity, Greek Method . = b
Average daily increase in acidity, A.O.A.C. Method = c

Using the observed rates of change in acidity as recorded in Table II and expressed graphically in Figure 1, we have computed and recorded in Table III the rate of change in acidity at 25° C.

and found that it would only require from 43 to 53 days for these several qualities of flour to reach the maximum permitted when the flours are stored at 25° C.

As was shown in the first report by Fifield and Bailey (1929) the rate of change is considerably increased when the temperature is elevated; thus storing these flours at 30° C. instead of 25° C. will probably result in the acidity reaching the maximum in 80% to 85% of the time required at 25° C. This would scarcely compensate for the tolerance of 10% that the Greek Government will permit in the acidity of flour from June 15 to September 15 of every year, at which time the average daily temperatures are considerably higher than during the remaining nine months.

Summary

A higher correlation of ash content with acidity as determined by the A. O. A. C. method was found in freshly-milled flours than of ash content with acidity determined by the Greek method.

Difference between Greek acidity and A. O. A. C. acidity in freshly milled flours was highly correlated with ash content.

Earlier assumptions that the A. O. A. C. tentative method for acidity indicated the relative rate of production of acid-reacting substances during the extraction with water received support from these recent studies.

Rate of change in acidity of flours stored at 25° C. was highly correlated with ash content.

It was estimated that flours containing the percentages of ash included in the four grades or qualities described in the present Greek specifications will contain in excess of the maximum limits of acidity after 43 to 52 days when stored at 25° C.

WHEAT AND FLOUR STUDIES XVII

FACTORS INFLUENCING THE VISCOSITY OF FLOUR-WATER SUSPENSIONS IV

THE EFFECT OF EXTRACTION WITH SOLUTIONS OF THE POTASSIUM AND SODIUM HALIDES[1]

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(Received for publication April 28, 1930)

Gortner, Hoffman and Sinclair (1928, 1929) have shown that when wheat flour is extracted with salt solutions, the quantities of protein extracted show wide variation depending on the flour, the salt, and the salt concentration used. This has led them to ask what salt and what concentration is to be used in extracting and determining the so-called globulin of wheat flour. Assuming that the non-gluten protein is the difference between the total protein and the sum of the gliadin and glutenin protein as determined by Grewe and Bailey (1927), it is shown by Gortner, Hoffman and Sinclair that the quantities of protein extracted from the same flour with different salts and different concentrations of salt may vary between 89 and 389% of this non-gluten protein. These percentages are equivalent to 17 and 73.9% of the total protein of the flour respectively. It appears, therefore, that salt solutions must extract considerable quantities of the gluten proteins. In order to determine which of these, gliadin or glutenin, was extracted by salt solutions, the work reported in this paper was done.

Experimental

According to the work of Sharp and Gortner (1923) glutenin is the protein chiefly responsible for the high viscosity of acidulated flour-water suspensions. A comparison of the viscosities of two suspensions, one of which has been extracted with distilled water only and the other with salt solutions, the salt also being subsequently extracted, would indicate whether glutenin was being removed by the extraction with salt solution. Experiments were therefore conducted following the exact procedure given by Gortner, Hoffman, and Sinclair (1929). According to their procedure, the flour is extracted three times with salt solution, the dissolved or peptized protein each time being separated from the residue by centrifugation. It was found that centrifugation could

[1]Published with the approval of the Director.

not be used for our purpose as the residue could not be brought into smooth suspension for the viscosity determination, the suspension always being filled with lumps. The next method used was, therefore, to extract first with 1 liter of the salt solution followed by two extractions with distilled water in order to remove the salt. The residue after these extractions was made up to 100 cc., poured into the cup of the MacMichael viscometer and after acidulation with 0.5 cc. of 20% lactic acid the viscosity was determined using a No. 30 wire. The results obtained through the use of this procedure are given in Table I. Tenth normal solutions of KI, KBr, KCl, and KF were used, as Gortner, Hoffman, and Sinclair had found that the quantities of protein extracted by solutions of these salts varied in a regular manner, KI solutions extracting the most and KF solutions the least. Eighteen gram portions of flour were used in every case. The data in Table I show that excepting the

TABLE I

VISCOSITY OF FLOUR-WATER SUSPENSIONS EXTRACTED WITH 0.1 NORMAL SOLUTIONS OF THE POTASSIUM HALIDES

Salt Solution Used For Extraction	Viscosity	Nitrogen Extracted	
		cgm.	%
Distilled Water	135	14.75	43.56
0.1N KI solution	173	19.40	57.29
0.1N KBr solution	180	18.45	54.49
0.1N KCl solution	175	17.30	51.09
0.1N KF solution	100	15.70	46.36

case in which the solution of KF was used for extraction, significantly higher viscosities were obtained when salt solutions were used for extraction than when distilled water alone was used. It was thought that the difference in behavior of the KF solution was due to the effect of the KF in preventing the phytase from liberating inorganic phosphates from organic combination. Since these phosphates were not rendered soluble they were not removed by extraction and remained in the flour suspension to depress the viscosity. In the final series of experiments, therefore, the procedure was modified so as at least partially to eliminate this effect of the fluorides. All the preparations were first extracted with 1 liter of water at 40° C. The purpose of this was, obviously, to allow the phytase to act unhindered, thereby allowing the removal of as much as possible of the natural salts of the flour before extraction with the salt solutions. An extraction with 500 cc. of salt solution was then made, followed by two extractions with distilled water using 1000 cc. each time. The last two extractions were ob-

viously to remove the salt. The residue after these extractions was made up to 100 cc. and the viscosity determined in the manner already indicated. The data obtained are given in Table II.

In these final experiments both the sodium and potassium halides were used, at concentrations of 0.5 and 1.0 N. The H-ion concentrations of all the suspensions were practically the same and therefore this factor can be eliminated as affecting any of the properties under consideration. The data in Table II show that all the flour suspensions extracted with salt solutions have higher viscosities than the suspension extracted with distilled water. It should perhaps be stated that the suspension extracted with distilled water received the same treatment as the other suspensions

TABLE II

THE VISCOSITY OF FLOUR-WATER SUSPENSIONS EXTRACTED WITH NORMAL AND 0.5 NORMAL SOLUTIONS OF THE POTASSIUM AND SODIUM HALIDES. THE QUANTITY OF NITROGEN EXTRACTED IS ALSO GIVEN

Salt Solution Used For Extraction	Viscosity Degrees MacMichael	Nitrogen Extracted	
		cgm.	%
Distilled Water	135	14.85	43.86
1.0N KI solution	199	17.02	50.27
1.0N KBr solution	179	16.58	48.96
1.0N KCl solution	169	16.15	47.66
1.0N KF solution	150	15.70	46.36
0.5N KI solution	189	17.60	51.98
0.5N KBr solution	182	17.32	51.15
0.5N KCl solution	178	16.98	50.14
0.5N KF solution	153	14.80	43.71
1.0N NaI solution	200	18.20	53.75
1.0N NaBr solution	198	17.40	51.38
1.0N NaCl solution	185	16.80	49.61
1.0N NaF solution	160	16.10	47.55
0.5N NaI solution	214	17.18	50.73
0.5N NaBr solution	186	17.00	50.20
0.5N NaCl solution	169	16.85	49.76
0.5N NaF solution	144	16.35	48.29

except that an extraction with distilled water replaced that with salt solution. The viscosity of the suspension extracted with distilled water was 135° while the viscosities of the suspensions extracted with salt solutions varied between 144° and 214° MacMichael. Of the suspensions extracted with salt solutions the viscosity was always highest for that extracted with the iodide solution and lowest for that extracted with the fluoride. Thus the viscosities of the solutions extracted with distilled water and with normal KI, KBr, KCl, and KF were 135°, 199°, 179°, 169°, and 150° MacMichael respectively. It appears, therefore, that the extraction of flour-water suspensions with solutions of the halides of

potassium and sodium leaves residues the viscosities of which after acidulation, vary in accordance with the lyotropic series of their anions. It is possible that the viscosity of suspensions extracted with the fluorides may be low because these salts inhibit the action of phytase. This is not believed to be the case, however, as this factor has been eliminated for the most part by the preliminary extraction with water, and as the viscosities obtained on extracting with the other salt solutions so closely parallel their effectiveness in peptizing flour protein.

Gortner, Hoffman and Sinclair (1928, 1929) noted that potassium salts had greater peptizing effects on the flour proteins than sodium salts and further that increasing the concentration of these salts decreased peptization. The data in this paper do not show significant differences in viscosity paralleling these differences in peptizing effect. This is probably because the peptizing effects on flour protein of different salt concentrations and different cations in the lyotropic series are not so pronounced as in the series of anions, and also because the viscosity determination is not sufficiently accurate.

The quantities of protein extracted by the different salt solutions were also determined and the data are given in Table II. The results agree with those of Gortner, Hoffman and Sinclair in that the quantity of protein peptized decreased in the order: iodide, bromide, chloride, fluoride, but do not agree in the absolute quantity peptized. Thus our data show that the extreme limits of peptization by iodide and fluoride were 53.75 to 43.71% of the total protein respectively. In the work of Gortner, Hoffman and Sinclair these limits were 73.9 and 10.3% respectively. This disagreement is probably due to differences in methods of procedure. Thus on the basis of an 18 g. sample, Gortner, Hoffman and Sinclair made three extractions using 150 cc. of salt solution each time, while in the work reported here the first extraction was made with 1000 cc. of water at 40° C., the second with 500 cc. of salt solution and the third and fourth with 1000 cc. of water.

Discussion

It appears that the extraction of flour-water suspensions with salt solutions produces suspensions whose viscosities after acidulation are higher than those of similar suspensions extracted with distilled water, even though more protein is extracted by the salt solutions than by distilled water. Since glutenin is the protein to which is due the high viscosity of extracted acidulated flour-water

suspensions, the higher viscosity of the suspensions extracted with salt solutions indicates that the increased protein removed was not glutenin. The 40 to 50% of the total protein removed by extraction with salt solutions must therefore have consisted largely of the alcohol-soluble protein of wheat flour. Sharp and Gortner (1923) and Johnson and Herrington (1928) have previously noted that when flour suspensions are more completely extracted by washing many times with distilled water and more protein (presumably gliadin) is removed, higher viscosities result. If the salt extraction removes gliadin at a more rapid rate than distilled water extraction it is obvious that the suspension extracted with salt solution will have the higher viscosity.

The fact that extraction of a flour-water suspension with a salt solution causes high viscosities of the salt-free acidulated suspension has previously come to our attention. Thus Johnson and Green (1930) have shown that in flour-water suspensions containing yeast and sodium chloride, the salt not only prevented the yeast from affecting the protein so that it no longer would imbibe water on acidulation, but when the salt was removed the acidulated suspension had a higher viscosity than a similar suspension not containing salt and yeast.

Conclusions

The viscosities of flour-water suspensions extracted with 1.0 and 0.5 N solutions of the potassium and sodium halides are higher tnan those of similar suspensions extracted with distilled water. The viscosities decrease according to the lyotropic series of the anions I>Br>Cl>F. Glutenin is not removed on extraction of a flour suspension with the halides of potassium and sodium.

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EFFECT OF OVERGRINDING OF FLOUR UPON THE KEEPING QUALITY OF BREAD[1]

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(Received for publication August 1, 1930)

During the baking of a bread dough the starch granules contributed by the flour are more or less converted into a highly concentrated gel. This involves an appreciable increase in the proportion of water imbibed by the starch. Our present knowledge of the phenomena associated with the development of staleness in the baked bread indicates that the principal process involved is the reformation of the starch granules of the amorphous stage from the gelatinized form with coincident loss of water. The process is reversible, but it would appear that the more that gelatinization of the starch is increased in baking, the more will the reformation of the amorphous "stale stage" be impeded.

In grinding wheat starch in a pebble mill, Alsberg and Perry (1924) found that all the grains were injured. The battered grains swell instantaneously when they come in contact with cold water and to considerable degree disperse themselves in it. Alsberg and Griffing (1925) reported that if flour was severely overground the baking quality was injured despite increased absorption, due to swelling of the starch granules. On the other hand, moderate overgrinding may injure the starch without noticeably affecting the gluten. Similar conclusions were drawn by Shollenberger and Coleman (1926). An increase in the cold water extract from the dispersed starch and an increased saccharogenic power were also observed as reported by Alsberg and Griffing (1925), Shollenberger and Coleman (1926), and recently by Pascoe, Gortner and Sherwood (1930).

Having been engaged in the investigation of the fermentation rate of overground flours the writers expanded these studies to determine whether or not the increased dispersion and the resulting increased reaction ability of the starch granules during fermentation and baking was reflected in the keeping quality of breads baked from overground flours in comparison to those made from the corresponding untreated samples.

Four different flours were used for these experiments:

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[1]Published with the approval of the Director as Paper No. 966, Journal Series, Minnesota Agricultural Experiment Station.
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No. 12428 Milled from a wheat mixture containing a high percentage of white wheat in Seattle, Washington; moisture 12.5%; ash 0.37%; crude protein 10.2%.

No. 12508 An 80% patent flour milled from a blend of spring wheats by the Minnesota State Testing Mill, containing 14.5% moisture, 0.45% ash and 11.7% crude protein.

No. 12778 Bleached straight grade flour milled from a blend of hard spring wheats by the Minnesota State Testing Mill, containing 13.5% moisture, 0.49% ash and 11.9% crude protein.

No. 12806 Straight grade spring wheat flour containing 12% crude protein.

The "overgrinding" of the flour was effected by first bolting out all particles that would pass through a 25xx flour silk, and then grinding the tailings twelve times, bolting after each grinding operation. A small portion of the flour was not reduced to the

TABLE I

VISCOSITY OF BREAD BAKED FROM OVERGROUND AND UNTREATED FLOUR

Flour No.	Treatment	Fresh t/t_1	After 42 hrs. t/t_1	Decrease of the viscosity in %
12428	Untreated	3.103	2.000	35.5
	Overground	3.074	2.012	34.5
12508	Untreated	2.730	1.936	29.1
	Overground	2.728	1.950	28.5
12778	Untreated	3.013	1.962	34.9
	Overground	2.938	2.037	30.7
12806	Untreated	2.949	1.936	34.3
	Overground	2.839	1.951	31.3

t is the time of flow of the suspension;
t_1 is the water equivalent of the viscometer (78 sec/5 at 20° C.)
The last column shows the decrease of the viscosity in per cent.

requisite fineness by this treatment, and it was ground in a porcelain mortar, bolted and added to the other material. All the fractions were then thoroughly mixed, and finally bolted three times through a coarse sieve.

The doughs were mixed using 300 g. flour, 7.50 g. sugar, 5.25 g. salt, 9.00 g. yeast and 6.75 g. shortening. After mixing, the doughs were divided in three equal parts and fermented at 30° C., according to the A. A. C. C. method. The first punch was made after 165 min. and the second after an additional 50 min. After a third period of 25 min. the doughs were panned and proofed for 55 minutes at 30° C. and were then baked for 25 min. at 230° C.

For measuring the march of staleness the viscosimetric method developed by Karacsonyi (1929) was used. Stated briefly, 10 g. of the crumb of the cooled loaf was forced through a 5xx silk sieve and after completing the weight to 100 g. with water and making the suspension uniform, its viscosity was determined by means of an Ostwald viscometer at 20° C.

After 42 hours storage, viscosity of crumb of the loaves was measured, compensating for the loss of water by evaporation in weighing off the charge for the determination.

In addition to the study of loaves made with overground flour, control loaves made with untreated flour were included in the series as a basis of comparison. The results of these observations are recorded in Table I.

The results obtained seem to indicate that overgrinding has but a very small effect upon the keeping quality of bread, so far as is disclosed by the method of study employed. The decrease of the viscosity of loaves made from overground and the corresponding untreated flours was almost the same in the case of the shorter patent samples. In the case of the two straight grade flours, however, the staling was slightly retarded.

These observations are in accordance with the results of the investigation conducted by the writers in order to detect differences, if any, in the gas-producing and gas-retaining ability of doughs made from overground and untreated flours. The results of the experiments referred to were essentially negative, and it may be concluded that by the method of overgrinding employed the starch granules were not modified to such an extent as to cause significant changes either in the fermentation rate of doughs or the keeping quality of the resulting loaves.

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RELATION OF PEPTIZATION OF WHEAT FLOUR PROTEIN TO LOAF VOLUME

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(Received for publication July 8, 1930)

The evaluation of baking strength of flour has been and still is a problem of some difficulty. The accepted method, that of making a laboratory baking test under rigidly specified conditions, has certain disadvantages: it is cumbersome, time-consuming, expensive and calls for a skilled operator. Blish (1928) mentions four supplementary tests in finally determining the full potentialities of a flour. Investigations published by Larmour and MacLeod (1929) have shown the advisability of using a formula including an "improver" in addition to the basic method. Clark (1929) stresses the value of a variable fermentation time. Harrel (1929) describes variations in loaf volume and characteristics, introduced by differences in panning, oven temperature control and other factors. To review all the literature published on this subject would be beyond the scope of this paper.

The chief aim of many investigators in the field of flour chemistry has been to find a method which would definitely place a flour in regard to strength without necessarily going through the baking procedure. Protein content has for a long period been considered the chief factor associated with baking strength, hence the practically universal demand for high protein wheat. It was believed by many workers that a quality factor must be present. This belief was based upon the fact that when a series of flours were baked, the results often varied from those forecasted from protein content alone. Differences of quality among the wheat proteins might explain these discrepancies. A brief survey of some of the physical and chemical methods applied to this problem follows.

Bailey and Le Vesconte (1924) measured the extensibility of a series of doughs with the Chopin extensimeter and concluded that these values were of use in indicating the strength or baking value of a flour. Gortner (1924) describes a method of measuring gluten quality by means of viscosity measurements and points out that the viscosity of flour-water suspensions is the resultant of two factors: quantity and quality of the glutenin present. A constant *b* was determined which appeared to be characteristic of glutenin quality. Two flours of widely different protein contents were found

to give almost identical loaf volumes but the flour with lower protein content gave much higher quality readings by the method described. Bailey (1924) employed this method as a means of determining the gluten quality of flour streams from the Minnesota State Mill and found substantial evidence of variation. Middlings flour rated highest and break flours lowest in gluten quality. Blish and Sandstedt (1925) found no adequate agreement between *b* as a measure of gluten properties and results of baking tests.

Gortner (1923) pointed out that flour strength, as far as gluten quality is concerned, is conditioned by colloidal properties of the glutenin, and found that changes induced in viscosity by addition of acid and alkali were very similar to those reported by Loeb for gelatin, casein, etc. Unlike Loeb, however, Gortner ascribed these changes to the force of surface phenomena, whereas Loeb viewed them in the light of chemical reactions.

As early as 1883, crude gluten was observed by Balland (1884) to change in weight and consistency when immersed in solutions of inorganic salts. Later, Wood (1907) dispersed gluten by the addition of acids to distilled water containing the gluten. This effect appeared to be largely counteracted by the addition of salts to the medium. Upson and Calvin (1915, 1916) weighed gluten discs after immersion in various solutions of acid and salts and determined the amount of imbibition of water. Their results tended to confirm the conclusions of Wood, that changes in gluten properties were due to environmental chemical variations rather than to inherent differences in the gluten itself.

Gortner and Doherty (1918) found a difference to exist between the gluten from strong and weak flours. Strong flour glutens tended to imbibe more water in dilute acid solutions, and dispersed less than weak flour glutens. They postulated that "the difference between strong and weak glutens is apparently that between a nearly perfect colloidal gel with highly pronounced physico-chemical properties, and that of a colloidal gel in which these properties are less marked."

Hoffman and Gortner observed (1927) that the extraction of flour with 5% potassium sulphate solution and 10% sodium chloride solution did not yield similar protein fractions, but that marked differences existed in the protein material dissolved by the solutions. Gortner, Hoffman and Sinclair (1928) studied the effect of 21 inorganic salt solutions on the proteins of 12 commercially milled flours of widely divergent origins. Striking differences in protein extracted were shown, both with a single salt solution and

different flours and a single flour and different salt solutions. Normal KF extracted 18.0% of the total protein in one flour, while normal KI extracted 73.9%. The KF values ranged from 10.5% to 18.0%, the KCl values from 18.7 to 30.4%, the KBr values from 30.1 to 48.6% and the KI values from 55.4 to 73.9%.

These characteristic variations suggested to Gortner the possibility of a correlation existing between the peptizing action of the salt solutions and the loaf volumes of the flours. Thirteen correlation coefficients were therefore calculated, ranging in magnitude from $r = -.672 \pm .107$ to $r = -.929 \pm .028$. The correlation coefficient between loaf volume and per cent total protein soluble in 5% K_2SO_4 for a series of flours was also computed by Gortner, and it was found that $r = -.824 \pm .065$. These results appeared to substantiate the conclusion that a relationship must exist between ease of peptization of the flour proteins and the baking strength, as registered by loaf volume.

The effect of the anions in causing peptization arranged themselves in a Hofmeister series of $F < SO_4 < Cl <$ tartrate $< Br < I$. The cations similarly were found to constitute a lyotropic series, rather less pronounced than the anions, in increasing order of peptization of $Na < K < Li < Ba < Sr < Mg < Ca$. These effects were found to be independent of H-ion differences, in opposition to the views of Loeb (1922).

Larmour and MacLeod (1929) found a significant negative correlation to exist between loaf volume and per cent total protein peptized by solutions of $MgSO_4$ and KBr. A somewhat different method from that used by Gortner was employed by these workers and will be discussed in detail later. It was shown by Gortner (1929) that the peptization of wheat flour proteins by inorganic salt solutions reveals the same sort of differences as does peptization with acid or alkaline solutions. He believes that these variations are tied up with the colloidal properties of the wheat flour protein, and are related to heritable differences as well as environmental factors during growth, harvest and storage of the original wheat.

Before proceeding to the study of the relationship existing between flour protein peptization and baking strength, it was decided to make a preliminary study of the methods of peptizing proteins in flour suspension and select a suitable procedure eliminating the necessity of employing a mechanical shaker and electric centrifuge.

The method used by Gortner and his co-workers was to weigh

out 6 g. of flour into a 100 cc. centrifuge tube, and to add 50 cc. of a salt solution. The mixture was then shaken for 30 min. in a mechanical shaker, and the flour residue tightly packed in the bottom of the tube by an electric centrifuge. The clear supernatant liquid was decanted into a Kjeldahl flask, and the extraction repeated on the flour residue with 50 cc. of fresh salt solution, repeating the shaking, centrifuging and decanting, and following this with a third extraction of the residue with a fresh 50 cc. portion of salt solution. The nitrogen dissolved by the salt solution was determined on the combined extracts by the usual Kjeldahl-Gunning procedure. These extractions were done in duplicate.

Larmour and MacLeod also used 6 g. of flour in their method, but weighed it into a 200 cc. glass bottle, which would just fit the larger cups of an electric centrifuge. The entire 150 cc. of salt solution was added at once, any adhering flour cleared from the bottom or walls of the bottle, and the bottle placed in a rotary motor-driven shaker. The mixture was shaken for 2 hours and the residual flour tightly packed in the bottom of the bottle by an electric centrifuge, and the supernatant liquid was decanted and aliquoted in two 50 cc. portions. These extracts were transferred to Kjeldahl flasks, and the nitrogen extracted determined as before.

In the first method, after the initial extractions, difficulty was experienced in breaking up the residual flour sufficiently to ensure thorough mixing of the residue with the fresh salt solution. Slight errors were necessarily introduced through removal of flour and solution adhering to the glass rod used in disintegrating the residue. It was also found that in several cases the mixture foamed quite badly during the third extraction and after centrifuging great care had to be exercised lest portions of the residual flour should disintegrate and pass with the clear liquid into the Kjeldahl flask. The second method was not open to these objections, and duplicate nitrogen determinations could be made on a single extraction, thus greatly reducing the work. It seemed probable, however, that a shorter extraction period would prove more desirable and that the results obtained would be relatively the same.

The first method described would be unsuitable for the purposes of the present study, a complete removal of liquid after each extraction necessitating an electric centrifuge, and a closely packed residue being necessary if all the solution were to be used for determining the peptized nitrogen. The second method seemed more suitable, and after a few trials a modification was evolved

embracing the principles of this procedure but with certain changes eliminating the use of a mechanical shaker or electric centrifuge.

This method was as follows: 6 g. of flour was weighed into a 250 cc. Erlenmeyer flask, 150 cc. of the salt solution was then added, the flask stoppered and shaken to ensure thorough mixing of the flour and liquid. The shaking was continued at intervals of approximately 5 min. for one hour, to prevent settling of the suspended particles. The flask was then set aside for several hours to allow the suspended flour particles to settle. At the end of the period, when settling out appeared to be complete, the clear supernatant liquid was decanted from the residual flour, aliquoted into two 50 cc. portions, and the extracted protein determined by the Kjeldahl-Gunning procedure.

To determine the relative values of these methods in protein extraction, a series of 11 experimentally milled 75% patent flours of the 1929 crop was chosen embracing a range in protein content from 9.0% to 16.0%. These flours were then peptized by the three methods, the extracted protein calculated in per cent total protein for each method, and the results correlated in pairs. The correlation between per cent total protein extracted and the "improver" loaf volume was also computed in each case. These correlations were as follows:

Method I and II, $r = +.9333 \pm .0262$	Method I and L.V., $r = -.8900 \pm .0423$
Method I and III, $r = +.8376 \pm .0607$	Method II and L.V., $r = -.7606 \pm .0857$
Method II and III, $r = +.7601 \pm .0859$	Method III and L.V., $r = -.8466 \pm .0576$

The Z transformation of Fisher was applied to the pair showing the largest difference in each set, and no significant difference was found. Therefore, any of the three methods are equally efficient for purposes of determining the peptizable protein, and its relation to baking strength. The third method, being the most convenient for the purposes of the present study, was adopted.

Total protein, improver loaf volume and per cent total protein peptized by the three methods using KBr as the peptizing agent, are shown in Table I. Method I gives consistently higher results in peptized protein, while methods II and III yield much similar values.

As the laboratory temperature fluctuated from day to day, it seemed desirable to obtain some data on the possible effect of temperature changes upon the peptization of wheat flour proteins by inorganic salt solutions. A series of 5 experimentally milled straight flours, of the following protein content, was peptized by

the third method, using 0.5N solutions of KF, KCl, KBr, KI and distilled water.

Sample No.	Protein Content %
946	8.0
917	9.8
98	12.6
935	17.7
927	18.3

Flasks containing the weighed flour and the solutions were brought to the correct temperature before starting, the solution was then added to the flour, and the flask held at the desired temperature until the expiration of the extraction period—one hour. Two temperature readings were taken from each series of 5 flours at intervals of 15 min. during peptization, and the maximum variation found to be $\pm 1°$ C. The 60° temperature run was done in an electric drying oven with thermostatic control, the lower temperature in an automatic proofing cabinet, with the regulator set at the desired point.

The data obtained, calculated as per cent of the total protein extracted, are shown in Table II. No significant change in protein extracted with rise in temperature is shown except a slight increase in the water extraction and two of the KI series. The increase in soluble protein in water at higher temperature is probably due to increased proteolytic activity. The behaviour of the two KI extractions is not followed by the other members of the KI group.

Another series of extractions was run to determine the influence of length of extraction period upon the percentage of total protein extracted. Two experimentally milled straight flours (No. 927, 18.3% protein and No. 946, 8.0% protein) were extracted with 0.5N solutions of KF, KI and water. These were run in series of five, one sample was thoroughly shaken immediately the solution was added, usually about 5 seconds was required for this. The other tests were made by agitating the mixture sufficiently, at intervals to ensure the flour remaining in suspension for periods of 5, 15, 30 and 60 min. each. The usual procedure of settling out, decanting, etc., was followed, according to the third method. The results obtained, computed as per cent total protein extracted, are shown in Table III, and depicted graphically in Figure 1.

No significant increase in extracted protein is evident after the first 30 min. except for the KI extracts, and here the change is relatively slight. The protein content of the flours does not appear to have a measurable effect upon the rate of extraction.

TABLE I

PER CENT TOTAL PROTEIN PEPTIZED BY EACH OF THE THREE METHODS FROM 11 FLOURS BY 0.5N KBr.

No.	Crude Protein %	Percentage of Total Protein Peptized			Improver Loaf Volume cc.
		Method I	Method II	Method III	
1	12.0	34.0	30.1	31.2	535
13	14.7	30.2	25.2	28.3	680
14	15.2	29.4	22.3	20.5	650
20	11.3	33.0	29.6	25.5	560
21	11.5	33.9	29.9	30.0	490
25	16.0	29.9	27.9	21.8	730
26	12.6	32.4	29.5	21.8	620
43	14.7	30.2	23.6	21.4	644
61	9.0	36.7	32.9	34.9	410
62	11.2	32.8	28.5	29.7	490
63	9.1	39.2	35.6	34.0	440

TABLE II

TABLE SHOWING PER CENT OF TOTAL PROTEIN EXTRACTED FROM FIVE FLOURS BY DIFFERENT SALT SOLUTIONS AND DISTILLED WATER AT VARIOUS TEMPERATURES

Flour No.	20° C.	38° C.	58° C.
	Distilled Water		
946	22.9	24.2	24.8
917	20.0	20.9	23.2
98	21.4	23.4	27.8
935	16.1	17.4	20.3
927	15.7	17.1	20.4
	0.5N KF		
946	19.9	22.4	22.5
917	17.2	20.3	18.6
98	14.8	14.6	16.0
935	12.5	13.5	13.2
927	12.5	14.7	13.8
	0.5N KCl		
946	28.2	28.6	27.3
917	24.2	24.0	23.7
98	20.4	20.7	20.2
935	16.9	17.8	17.1
927	17.8	19.2	17.8
	0.5N KBr		
946	38.0	38.8	37.4
917	34.8	33.7	35.0
98	35.0	31.6	31.9
935	26.9	31.4	28.8
927	28.3	27.9	28.5
	0.5N KI		
946	54.9	57.7	58.4
917	45.5	54.6	62.5
98	38.5	50.3	54.2
935	47.7	49.2	50.5
927	...	49.4	50.9

TABLE III

TABLE SHOWING PER CENT OF TOTAL PROTEIN EXTRACTED FROM FLOUR BY TWO SALT SOLUTIONS AND DISTILLED WATER, FOR DIFFERENT LENGTHS OF EXTRACTION PERIOD

Time (Min.)	Per Cent Total Protein Extracted by		
	H_2O	0.5N KF	0.5N KI
	Flour No. 946		
1/12	14.4	15.0	37.8
5	17.8	17.1	46.2
15	18.9	17.8	50.0
30	20.3	17.8	50.4
60	21.0	17.8	53.2
	Flour No. 927		
1/12	12.7	10.2	29.6
5	13.1	11.1	40.8
15	13.0	11.6	45.7
30	13.4	11.6	47.9
60	14.5	11.7	49.4

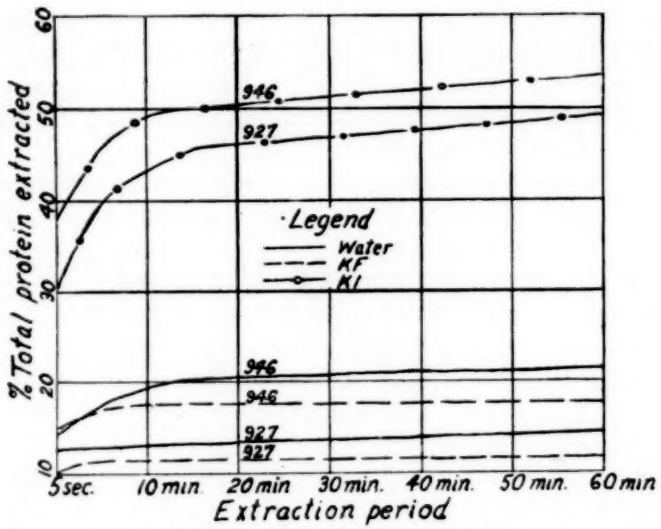

Fig. 1. Relation between per cent total protein extracted from two flours of different protein contents, and length of extraction period.

A series of wheats, 44 in number, from the 1929 Saskatchewan wheat crop, were experimentally milled into 75% patent flour on an Allis Chalmers experimental mill, and the resultant flours baked and analyzed. These wheats were all of the Marquis variety, and of contract grade, except one sample, No. 25, and were grown in the farming territory near Saskatoon. No frost or other damage was apparent in any of these samples and all would be considered satisfactory milling wheat.

After bleaching with a dosage equivalent to one pound reagent

to forty-five barrels of flour, these flours were baked by two methods, one using the basic formula employing flour, sugar, salt and yeast, and another in which an improver (1% malt + .001% $KBrO_3$, or for a relatively high protein flour, 3% malt + 0.5% Arkady) was added to the basic ingredients. They were peptized according to the third method, already outlined, by 0.5N solutions of KBr and $MgSO_4$, and the per cent total protein removed was calculated. These values, with the corresponding loaf volumes obtained by the two baking methods and the total protein, are shown in Table V. [Note: The author's paper, "Relation between crude protein content and loaf volumes obtained by two different methods of baking" (Cereal Chem. **7**: 557-570, 1930), in Tables I and II, data are shown for 59 samples of wheat and flour, which include the 44 samples reported in the present paper.]

Figure 2 is a dot diagram of the flour protein content and the loaf volume obtained by the basic method, the correlation in this series of 44 being $r = +.6937 \pm .0527$. Figure 3 is a dot diagram of the same flour showing protein content and loaf volumes obtained with use of improver formula. Here the correlation is $r = +.9021 \pm .0189$. A comparison of the two diagrams shows a regular distribution of the dots along the regression line in the latter, and a high probability of predicting loaf volume from protein content. Figure 2 shows much more scattering in the distribution and a tendency to run parallel to the base axis. From a protein content of 12% to 16%, there is no significant increase, in contrast to the data of Figure 3, where a steady rise of loaf volume with increasing protein content is shown.

Figures 4 and 5 show graphically the percentage of total protein peptized by 0.5N KBr, and the loaf volumes obtained with the two baking methods. In the basic method shown in Figure 4, $r = -.4579 \pm .0804$. While this shows a relationship between flour strength as registered by loaf volume and per cent total protein extracted, it does not indicate any great probability of accurately predicting loaf volume from degree of protein peptization. Figure 5, a diagram of the improver loaf volumes and KBr extracts, yields a correlation of $r = .7326 \pm .0471$, and affords a much greater probability of predicting loaf volume although much scattering is evident.

Figures 6 and 7 represent 0.5N $MgSO_4$ extracts in a similar manner, these data giving correlations of $r = -.4789 \pm .0784$ and $r = -.6148 \pm .0632$ with the basic and improver loaf volumes

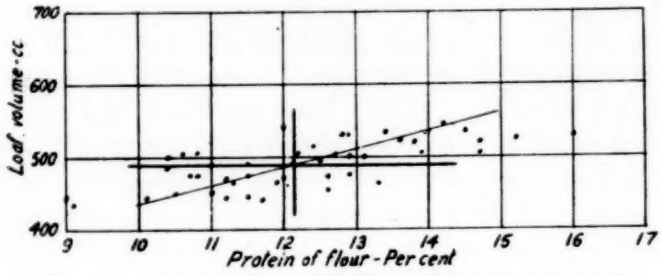

Fig. 2. A dot diagram of Protein of Flour Per Cent and Loaf Volume (Basic Formula).
$n=44,\ r=+.6937\pm.0527$

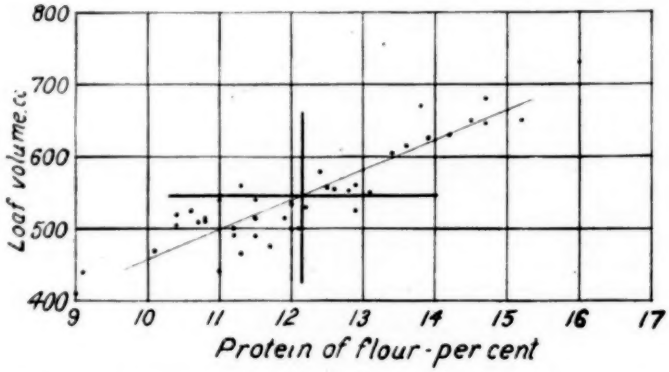

Fig. 3. A dot diagram of Protein of Flour Per Cent and Loaf Volume (Improver Formula)
$n=44,\ r=+.9021\pm.0189$

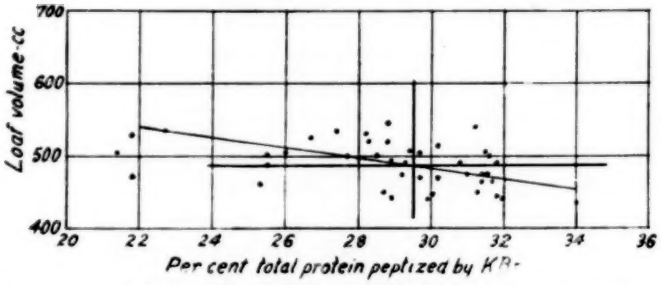

Fig. 4. A dot diagram of Per Cent Total Protein Peptized by KBr and Loaf Volume. (Basic Formula)
$n=44,\ r=-.4579\pm.0804$

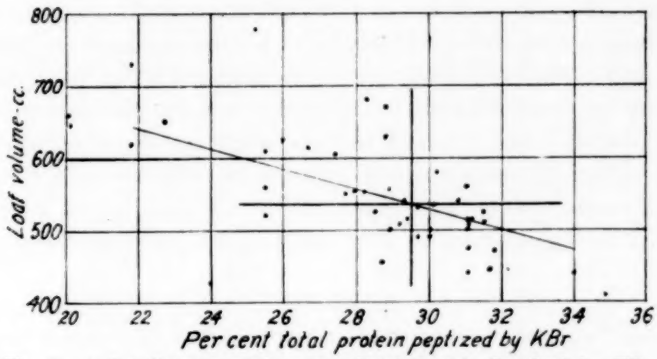

Fig. 5. A dot diagram of Per Cent Total Protein Peptized by KBr and Loaf Volume, (Improver Formula)
$n=44$, $r=-.7326\pm.0471$

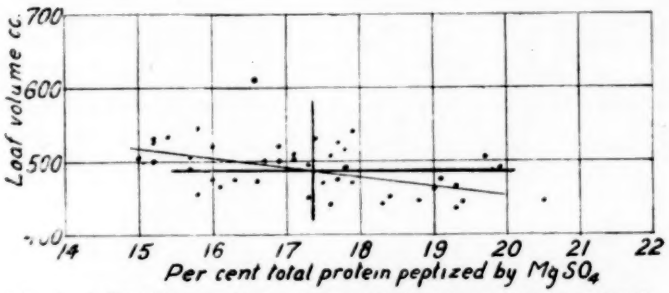

Fig. 6. A dot diagram of Per Cent Total Protein Peptized by $MgSO_4$ and Loaf Volume (Basic Formula)
$n=44$, $r=-.4789\pm.0784$

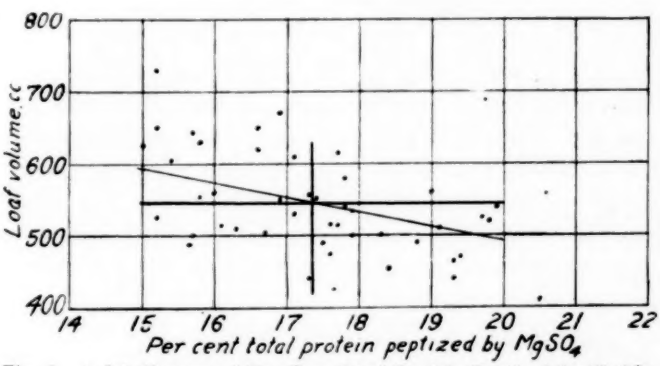

Fig. 7. A dot diagram of Per Cent Total Protein Peptized by $MgSO_4$ and Loaf Volume (Improver Formula)
$n=44$, $r=-.6148\pm.0632$

respectively, and show as in the case of the protein contents and KBr extracts, more symmetrical distribution with the improver.

A further statistical study, using the method suggested by Geddes and Goulden (communication received from W. F. Geddes), was made of these 44 samples, and the results are shown in Table IV. The data when treated in this manner, show strikingly the greater value of non-peptized protein in relation to loaf volume. Peptized protein does not appear to be of very great significance as shown in its relation to loaf volume and non-peptized protein.

TABLE IV

STATISTICAL CONSTANTS COMPUTED FROM THE PEPTIZATION AND BAKING TEST DATA 12 FLOURS OF 1928 CROP—44 FLOURS OF 1929 CROP

	KBr			$MgSO_4$		
		Basic	Improver		Basic	Improver
r_{ae}	=	$+.6937 \pm .0527$	$+.9021 \pm .0189$	r_{ae} =	$+.6937 \pm .0527$	$+.9021 \pm .0189$
r_{ad}	=	$-.7165 \pm .0495$		$r_{ad'}$ =	$-.6209 \pm .0625$	
r_{be}	=	$+.3667 \pm .0880$	$+.2585 \pm .0949$	$r_{b'e}$ =	$+.3908 \pm .0862$	$+.3866 \pm .0865$
r_{bc}	=	$+.2390 \pm .0959$		$r_{b'c'}$ =	$+.3014 \pm .0925$	
r_{ce}	=	$+.6640 \pm .0569$	$+.9128 \pm .0171$	$r_{c'e}$ =	$+.6735 \pm .0556$	$+.8921 \pm .0208$
$_bR_{ce}$	=	$+.6379 \pm .0603$	$+.9073 \pm .0181$	$_{b'}R_{c'e}$ =	$+.6331 \pm .0609$	$+.8820 \pm .0226$
$_cr_{be}$	=	$+.2863 \pm .0932$	$+.1015 \pm .1006$	$_{c'}r_{b'e}$ =	$+.2664 \pm .0945$	$+.2732 \pm .0941$
$R_{(bc)e}$	=	$+.6875 \pm .0536$	$+.9132 \pm .0169$	$R_{(b'c')e}$ =	$+.7017 \pm .0725$	$+.9006 \pm .0193$

Note:

a	= total protein
b	= peptized protein by 0.5N KBr
$c_{(a-b)}$	= non-peptized protein by 0.5N KBr
d	= % total protein peptized by 0.5N KBr
e	= loaf volume
b'	= peptized protein by 0.5N $MgSO_4$
c'	= non-peptized protein by 0.5N $MgSO_4$
d'	= % total protein peptized by 0.5N $MgSO_4$

A study was also made of 12 samples of pure strain Marquis wheat grown in 1928 in various parts of Saskatchewan. A description of these is found in Table VI. They include grades from No. 1 Northern to No. 6 and a protein content ranging from 9.8% to 14.6%. They were milled, analyzed and baked in the same way as the 44 samples already discussed, except that 1% malt and 0.001% $KBrO_3$ only were used as an improver. The baking and analytical data are reported in Table VII.

After milling and baking, these flours were peptized by 0.5N solutions of KBr and $MgSO_4$. The percentage of total protein extracted in these solutions is shown in Table VIII, which also includes the loaf volumes and protein contents of the flours. The correlations obtained are shown in Table IX. Inspection of this

TABLE V

PROTEIN CONTENT, LOAF VOLUMES AND PERCENTAGE OF TOTAL PROTEIN EXTRACTED IN HALF NORMAL SOLUTIONS OF KBr AND $MgSO_4$ FOR FLOURS MILLED FROM MARQUIS WHEAT OF 1929 CROP

No.	Crude Protein	Loaf Volume, cc.		Protein Extracted, %	
		Basic	Improver	KBr	$MgSO_4$
	%				
1	12.0	540	535	31.2	17.9
2	11.5	475	515	31.4	17.7
3	11.0	450	440	31.3	17.3
4	12.6	455	555	27.9	15.8
5	11.9	465	515	31.4	16.1
6	12.0	470	500	30.2	17.9
7	11.7	440	475	31.9	17.6
8	12.1	490	500	31.8	15.7
9	12.9	475	560	31.0	16.0
10	11.3	465	465	31.7	19.3
13	14.7	520	680	28.3	16.0
14	15.2	525	650	20.5	15.2
15	10.5	450	455	28.7	18.4
16	13.1	500	550	27.7	16.9
17	10.1	445	470	31.8	19.4
19	11.2	443	500	28.9	18.3
20	11.3	463	560	25.5	19.0
21	11.5	446	490	30.0	18.8
22	12.9	500	525	28.5	15.2
23	10.4	487	520	25.5	19.8
24	12.7	502	610	25.5	17.1
25	16.0	529	730	21.8	15.2
26	12.6	472	620	21.8	16.6
28	10.4	500	505	31.6	16.7
29	13.8	520	670	28.8	16.9
31	13.6	525	615	26.7	17.7
32	11.5	490	540	30.8	17.8
33	10.6	505	525	31.5	19.7
34	10.7	475	510	31.5	19.1
35	14.2	545	630	28.8	15.8
36	13.4	534	605	27.4	15.4
37	11.0	490	540	29.3	19.9
38	10.8	507	516	29.4	17.6
39	12.8	530	552	28.2	17.4
40	12.2	505	530	29.7	17.1
41	12.5	495	558	28.9	17.3
42	12.4	515	580	30.2	17.8
43	14.7	505	644	21.4	15.7
44	14.5	535	650	22.7	16.6
61	9.0	445	410	34.9	20.5
62	11.2	470	490	29.7	17.5
63	9.1	435	440	34.0	19.3
64	10.8	475	510	29.2	16.3
65	13.9	505	625	26.0	15.0

TABLE VI

DESCRIPTION OF WHEAT SAMPLES (1928)

No.	Grade	Weight per Imperial Bushel, lbs.	Crude Protein (13.5% moisture basis) Per Ct.
15	No. 2 Northern	66	14.1
16	No. 5	63½	13.9
17	No. 6	55½	14.3
18	No. 2 Northern	63	10.5
19	No. 4	65	12.8
20	No. 4	62	12.8
21	No. 4	64½	9.8
22	No. 4	63½	14.6
25	No. 2 Northern	64	11.6
26	No. 3 Northern	66	10.8
27	No. 1 Northern	66	14.4
28	No. 1 Northern	66	13.0

TABLE VII

BAKING AND ANALYTICAL DATA OBTAINED ON FLOURS MILLED FROM PURE STRAIN MARQUIS WHEATS OF 1928 CROP

No.	Crude Protein	Ash	Moisture	Water Absorption	Loaf Volume	Scores				Baking Score
						Color	Grain	Texture	Symmetry	
	%	%	%	%	cc.					
					B[1] 557	17	7	8	9	97
15	12.9	0.51	13.8	62	I[2] 625	20	9	8	10.5	109
					B 610	16	9	9	10	105
16	12.8	.46	13.5	63	I 615	18	10	9	10.5	108
					B 605	11	6	7	10	94
17	12.8	.545	13.9	64	I 595	12	8	7	10	96
					B 515	15	8	8	6	88
18	9.5	.510	13.0	62	I 531	15	9	9	7	93
					B 505	16	7	9	8	90.5
19	12.0	.465	12.9	63	I 562	17	9	8	9.5	99.5
					B 510	15	8	8.5	8	90.5
20	11.9	.420	12.9	63	I 557	18	9	8	9	99
					B 477	17	8	8	7	88
21	8.8	.425	12.9	63.5	I 470	19	9	7	6	88
					B 590	22	10	10	11	112
22	12.7	.315	12.7	63	I 630	22	11	9	11	116
					B 530	20	10	9	7	99
25	10.6	.435	13.5	63.5	I 490	16	6	8	5	84
					B 517	19	9	9	6	95
26	9.7	.425	13.7	63.5	I 510	18.5	7	8	4	88
					B 522	16	7	7	7	89
27	13.2	.335	12.7	61	I 630	21	10	9	11	114
					B 522	18	8	8	5	91
28	11.8	0.387	12.7	61.5	I 540	17	10	10	9	100

[1] Basic.
[2] Improver.

table shows a similar relation when the loaf volumes obtained by the two baking methods are correlated with the protein of flour and total percentage of protein peptized by KBr and $MgSO_4$.

TABLE VIII

TABLE SHOWING PROTEIN CONTENT, LOAF VOLUMES AND PERCENTAGE OF TOTAL PROTEIN EXTRACTED IN HALF NORMAL SOLUTIONS OF KBr AND $MgSO_4$ FOR 12 FLOURS OF 1928 CROP.

No.	Crude Protein	Loaf Volume		Protein Extracted, %	
		Basic	Improver	KBr	$MgSO_4$
	%	cc.	cc.		
15	12.9	558	620	32.5	19.2
16	12.8	610	615	33.1	19.4
17	12.8	605	595	36.0	22.0
18	9.5	515	531	37.2	23.6
19	12.0	505	562	33.5	21.0
20	11.9	510	557	33.7	20.7
21	8.8	477	470	36.7	24.4
22	12.7	590	630	32.7	19.5
25	10.6	530	490	34.4	21.9
26	9.7	517	510	36.0	22.1
27	13.2	522	630	31.6	19.9
28	11.8	522	540	32.9	20.1

TABLE IX

CORRELATIONS BETWEEN PROTEIN CONTENT, PERCENTAGE OF TOTAL PROTEIN PEPTIZED, AND LOAF VOLUMES BY TWO BAKING METHODS

No. Samples	Baking Method	Correlated With	Correlation	Probable Error	r_{xy}/P. E.	P.
12	Basic	Protein of flour	+ .7026	± .0986	7.13	<.02
	Improver	Protein of flour	+ .9074	± .0344	26.38	<.01
12	Basic	KBr Extract	— .2723	± .1803	1.51	>.1
	Improver	KBr Extract	— .6374	± .1156	5.51	<.05
12	Basic	$MgSO_4$ Extract	— .5411	± .1377	3.92	<.1
	Improver	$MgSO_4$ Extract	— .7974	± .0709	11.24	<.01

Summary

A series of 11 experimentally milled flours were peptized by three methods, and the per cent total protein extracted computed. The results were taken in pairs and the correlation coefficient calculated. The correlations between improver loaf volume and per cent total protein peptized were also computed. The differences between these coefficients were not found to be significant, when the Z transformation of Fisher was used. The effect of temperature and length of extraction period upon per cent total protein extracted by the method used in this study was investigated. The quantity of protein removed was found to increase quite rapidly at first, but no appreciable change was detected after the initial 30

minutes. Temperature did not appear to have any significant effect upon the quantity of protein extracted, except in the case of the water suspension, when a slight increase with rise in temperature was noticed.

Forty-four flours of 75% extraction, milled from 1929 crop Saskatchewan Marquis wheat of contract grade, were peptized by 0.5N solutions of KBr and $MgSO_4$, and the protein extracted calculated as per cent of the total protein, also as peptized and non-peptized protein fractions of the flour. Correlations between per cent total protein peptized and improver loaf volume, were lower than between total protein and improver loaf volume, and show less probability of forecasting loaf volume. The relationship between non-peptized protein and improver loaf volume is not significantly different from that involving total protein and improver loaf volume, and is of equal value in predicting baking strength. The corresponding correlations obtained with the basic loaf volume were not so large, and are relatively of less practical importance.

Further, 12 flours milled in a similar manner from 1928 pure strain Marquis, grown at various Saskatchewan points including grades from No. 1 Northern to No. 6, gave a higher negative correlation between per cent total protein peptized and improver loaf volume than between per cent total protein peptized and basic loaf volume.

Acknowledgment

The author wishes to thank Dr. R. K. Larmour of the University of Saskatchewan for his counsel and advice, and the Chemistry Department of the University of Saskatchewan for extending to him the facilities of their Cereal Laboratory.

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PROGRESS REPORT OF THE COMMITTEE ON STANDARDIZATION OF LABORATORY BAKING

C. H. BAILEY, *Chairman*

(Received for publication January 5, 1931)

The Committee on Standardization of Laboratory Baking convened at University Farm, St. Paul, Minnesota, on January 5, 1931, with six of the members of the committee present.

Mr. Paul P. Merritt, Research Fellow of the American Association of Cereal Chemists, presented a report of progress of the work

conducted during the period up to December 31, 1930. This report follows. It was discussed at some length by the committee, and was then approved, and it was voted that it be published in the January issue of Cereal Chemistry.

Following the acceptance of the report, the outline of the work to be conducted next was discussed at length, and it was agreed that detailed attention would be given to the items of mechanical mixing procedure and to the characteristics of bread produced by baking in different types of ovens.

Preliminary Report of A. A. C. C. Research Fellow on Experimental Baking Test

P. P. MERRITT

(Submitted January 5, 1931)

At the time that the Research Fellow reported at the Nebraska Experiment Station on September 1, 1930, the baking laboratory was not quite ready for operations. This was due in part to alterations that were to be made in the electric wiring, as necessitated by special current requirements of the several pieces of equipment. The new proofing cabinet and moulder arrived on September 2, 1930, and some further delay was occasioned incident to their installation and to the tuning up and calibrating of the temperature controls in the cabinet.

Considerable time was occupied in preliminary work incidental to the installation and adjustment of the various items of equipment, and in becoming familiar with the operation of these devices. The adjustment of the moulder required rather extensive preliminary work. The same was true to a lesser degree in the instance of the ovens. The new spotless metal pans that had been purchased for this work had to be "broken in" by heating them a number of times before they could be used with satisfaction. Thus, in the first attempts to use these pans, large holes frequently appeared near the bottom of the loaf, and the loaves likewise tended to draw away from the pans, particularly at the bottom. This trouble gradually disappeared with the extended use of the pans and most especially with frequent light greasing of the pans.

The flours used in these preliminary studies included a 95% bleached bakers flour, milled by a Nebraska milling company and

designated as Nebr. Flour No. 1. Later another similar flour was secured from the same source, which is designated as flour No. 2. Two samples of flour were obtained from a Minneapolis mill, one of them being bleached (flour No. 3) and one of them unbleached (flour No. 4). The results of the analysis of these four flours were as follows:

Flour	Ash	Moisture	Crude Protein
	%	%	%
1	0.45	13.76	11.29
2	.45	13.00	11.20
3	.47	13.66	14.76
4	0.47	13.40	14.82

Flour No. 2 was bleached less heavily than Flour No. 1, and gave larger loaf volumes. The Minneapolis flours had a high content of protein and gave loaves of large volume. In each instance the flours were stored for 6 weeks before being used, and were then placed in tin lard cans, each holding about 35 lbs. of flour, and these were thereafter stored at 0° C. until time for withdrawing sub-samples for testing purposes.

Yeast was delivered in pound lots on Wednesday and Saturday, and kept in an ice box. After a brief experience, it was kept on ice until just before mixing time for each dough. Salt and sugar were used in solution, 15 cc. of which contained 2.5 g. sugar and 1 g. salt. Hand mixing was done in a round bottom bowl slightly larger than the fermentation bowls, and with a limber spatula. Machine mixing was done in a Fleischmann mixer, the bowl of which makes 92 r.p.m., and the pins 169 r.p.m. 500 g. flour was used in machine mixing and the dough immediately scaled off into 5 equal parts.

The first undertaking was to compare hand with machine mixing, using Flour No. 1, and later with the other flours. Flours 3 and 4 acted alike, having almost the same volume, so the bleached flour was used in most of the work, more of it being available. Considering only Flour No. 1, hand mixing gave the following averages:

Standard Deviation, 10.02. Coefficient of Variation, 2.08.
Average Volume, 474 cc. Variates 160.

Machine mixing (1½ minutes) gave:

Standard Deviation, 10.49. Coefficient of Variation, 2.12.
Average Volume, 494 cc. Variates, 210.

Of the two methods of mixing, machine mixing gave the greater loaf volume with all flours, regardless of whether the mix-

ing period was 45 seconds or 3 minutes, or any period within that range. The external characteristics were the same except that the break was smaller in the hand mixed doughs. Internal characteristics of grain and texture could not be distinguished one from the other.

Machine mixing varying the mixing period was carried out using 45 seconds, 1 minute, 2 minutes and 3 minutes respectively. A mixing delay occasioned by the pins kicking the ball of dough around in the bowl was noticed, and it averaged about 20 seconds. The loaf volume increased with the lengthening of the mixing period. Variability was least at two minutes, which was thereafter used as a standard mixing period, and greatest at the extreme periods, due in the case of the 45 second period to unequal mixing and probably due to more or less gluten development in the case of the 3 minute period. External characteristics were practically the same over the entire range. The grain was constant, with a slight improvement in texture as the period was lengthened.

DATA ON MACHINE MIXING

Time	Standard Deviation	Coefficient of Variation	Average Loaf Volume	Variates
			cc.	
45 sec.	15.21	3.09	490	90
1 min.	11.64	2.37	493	85
2 min.	10.30	2.01	507	90
3 min.	17.49	3.32	525	60

Absorption studies on Flour No. 1 were made, varying the absorption by 2 cc. intervals from 58 cc. to 66 cc. per 100 g. flour. All loaves were hand mixed and machine moulded. The averages follow:

Water	Standard Deviation	Coefficent of Variation	Average Loaf Volume	Variates
cc.			cc.	
58	8.98	2.00	437	16
60	6.57	1.47	446	16
62	9.65	2.07	461	16
66	7.73	1.61	469	16
64	7.66	1.64	464	16

Internal and external characteristics were practically the same in variability, and 64 cc. per 100 g. flour were thereafter used with all flours.

The effect of greasing the pans daily in comparison with baking successively in ungreased pans was studied, using all four

flours. Greased pans showed a standard deviation of 7.6, coefficient of variation 1.41, and average loaf volume of 512 cc. Dry pans showed standard deviation 8.61, coefficient of variation 1.77, and loaf volume 515 cc. Greasing did not significantly influence loaf volume of other characteristics, and eliminated the trouble from holes and heaving up from the bottom of the loaves. Variates, 55 on each.

Hand versus machine moulding on three flours, with both hand and machine mixed doughs was studied.

Treatment	Standard Deviation	Coefficient of Variation	Average Loaf Volume	Variates
			cc.	
Hand moulded, Hand mixed	11.38	2.01	518	60
Hand moulded, Machine mixed	18.89	3.20	563	60
Machine moulded, Hand mixed	13.02	2.65	493	60
Machine moulded, Machine mixed	14.32	2.56	526	60

In this series, the hand-mixed loaves exhibited lower variability and loaf volumes were smaller than the machine-mixed loaves, regardless of the method of moulding. With all four flours, hand moulding gave larger loaf volumes than machine moulding where the doughs were mixed by hand. When machine mixed, hand moulding produced larger loaf volumes than machine moulding with the Minneapolis high protein flour, but with the weaker flour the advantage in volume was in favor of machine moulding.

A few tests involving adjustments of the moulder of rolls at 2, and the shoe at 1 11/16 inches measured vertically from exit end of the shoe to drum, gave the optimum loaf volume with fair uniformity as to external and internal characteristics. All previous moulding had been done with this setting. However, it was considered desirable to measure the effect of varying the setting of the compression plate in relation to the drum. In the following table the different settings are indicated as the distance from the exit end of the compression plate vertically to the drum:

Distance Inches	Standard Deviation	Coefficient of Variation	Average Loaf Volume	Variates
			cc.	
1 11/16	13.67	2.34	582	60
1 10/16	13.76	1.44	535	60
1 9/16	6.98	1.44	474	60

There was some loss in volume in passing from 1 11/16 to 1 10/16 inches, but a far greater reduction in reducing the setting

from 1 10/16 to 1 9/16 inches. These reductions in volume were featured by progressive lack of oven spring, and a progressively coarser grain, which was more noticeable in the weaker than in the stronger flours.

For the purpose of estimating the magnitude of the probable error of a single measurement of loaf volume, a wooden loaf model was measured 20 times. The standard deviation was 3.18, indicating a probable error of plus or minus 2 cc.

Summary

Hand mixing by a single operator gave smaller loaf volume and less variability than machine mixing with all four flours studied, with but slight differences in other characteristics. Lengthening the mixing period increased loaf volume progressively up to three minutes of mixing with the Fleischmann machine.

In absorption studies loaf volume increased progressively with additions of water from 58 to 66 cc. per 100 g. flour with Nebraska Flour No. 1.

With spotless metal pans, light greasing produced no significant changes in loaf volume or other characteristics, but reduced variability slightly.

In a comparison of hand moulding and machine moulding, the results are dependent upon the flour. With the stronger flours hand moulding resulted in larger loaves than did machine moulding in the instance of both hand and machine mixed doughs. Doughs mixed by the machine exhibited a greater variability, however, due to "bucky" doughs. With the weaker flours, hand moulding yielded larger loaves than machine moulding in the instance of hand mixed doughs, but with the machine mixed doughs the loaves were larger when machine moulded than when moulded by hand, although there was greater variability among the former.

In a study of the adjustment of the shoe of the machine moulder, it was observed that the loaf volume decreased progressively as the shoe was brought closer to the drum in effecting adjustments ranging from 1 11/16 to 1 9/16 inches, the greatest difference being effected through the range between 1 10/16 to 1 9/16 inches. The characteristics of the finished loaf were modified somewhat with these adjustments of the shoe, particularly as to grain and texture of the crumb in the instance of the weaker flours (No. 1 and No. 2). Variability in loaf volume was greatest in those instances where the loaf volumes were large in these comparisons.

BIOMETRIC ANALYSIS OF CEREAL-CHEMICAL DATA
I. VARIATION

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(Received for publication January 7, 1931)

Introduction

In the rapid evolution in comparatively recent times of the biological sciences from the stage of simple descriptive recording of observations concerning organisms and vital processes, through the period of comparative studies of increasing refinement to the present wide application of the experimental method there has been a constant transference of ideas inspired by and derived from the more highly developed systems of thought and technique of the maturer sciences. A worthy portrayal of the action of the influences that have stimulated sensitive responses in the master minds of biological science would form a most fascinating and truly invaluable series of volumes in the annals of human achievement. Merely to reflect upon the impregnation of mathematical thought into the reasoning processes today in biological and sociological research challenges one with a sense of realization of the molding forces at play.

It was entirely logical with the advent of the experimental method as a major technique in biological investigation, that "the attempt should be made to interpret biological phenomena in terms of the more highly developed sciences of physics and chemistry . . . in which it has long been realized that progress depends upon the exactness of the control of the conditions of experimentation, the precision of the measurements and the adequacy of the mathematical description and analysis of the measurements which have been made" (Harris, 1928). The "penetration of the mathematical leaven into the biological lump" has received a tremendous stimulus in our own times from a school of workers who were cradling their profound and difficult science at the time that cereal chemistry was coming into being as a prominent branch of the fast-growing phytochemical tree. Under the initial leadership and inspiration of Francis Galton a coterie of workers in England devoted themselves to the task of developing "mathematical formulae suitable for the analysis of the highly variable data of biological observation and measurement." Their science, Biometry, under the leadership of Karl Pearson, today represents perhaps the most

profound contribution to the progress of Biology in many past decades, for the small importance of the individual in determining a biological or sociological law is recognized by all of wide experience as making necessary the mathematical study of large numbers in these fields for safe interpretation of observations.[1]

An ever growing experience has already convinced most cereal chemists that the highly controlled conditions of experimentation available to the chemist and physicist studying elements and matter can only in rare cases be simulated in his laboratory. Whereas the amount of chlorine to be liberated by electrolysis from pure hydrochloric acid may be determined with a high degree of exactitude in a single trial, the fermentation power of a yeast preparation or even the amount of ash in a sample of flour can only be approximately learned from a single analysis, and many trials must be made before precision comparable to that of the physical chemist is attained. To be sure, precision is a relative term, and with the weapons of controllable electronic movements available to them, physicists today are learning that what was once regarded as absolute is really in most cases likely to be a variation system of a range that no longer remains infinitesimal. Thus the mathematics of probability, which plays a major role in biometric analysis, is making and will make increasingly a profound contribution to the so-called exact sciences, simply dealing with very much smaller quantities than comprise the variation of the results which the cereal chemist must interpret.

It is a matter in which cereal chemists may justifiably take some measure of pride that the precision methods of statistical treatment early found an important level in the analysis of problems by her pioneer workers. The work of Roberts (1910, 1921) and Zinn (1923) with univariate and bivariate distribution studies did much to raise the standards of interpretation of cereal data beyond the attack of criticism from the point of view of prejudiced and unwarranted deductions. No other large group of chemists has faced the problem of establishing standards of accuracy in analytical work with the frankness and practical approach of the statistical analysis of *actual results* as has been done on a wide collaborative scale in the cereal laboratories of the North American continent. True, only the beginning has been made and tremendous tasks yet lie ahead, but that start has much of priority in it that other biological and chemical workers may emulate.

[1] In marked contrast is the serviceability of any simple gas for the establishment of Boyle's Law, or the hypothesis of Avagadro, or other profound laws of chemistry and physics.

The progress of theory in biometric analysis has far outstripped the wide application that should follow most closely to test the universality of the theorists' deductions. This is in part an unfortunate consequence of modern specialization, and partly of a certain apathy of workers in biological fields toward the understanding of reasoning processes that demand effort of a type novel to them. This situation is one which spells a severe loss to both groups and retards the advance of scientific precision in the fields effected. Believing that the deficiency may, in some measure, be remedied by "border-line" workers undertaking the tasks of interpretation, the present series of articles is presented in an endeavor to trace, in a most simple form, the elementary groundwork of Biometry as it finds application in the field of cereal chemistry data. It is hoped that the large group who find the customary mathematical text of little aid will in this way be rendered some service, offsetting the criticism of the more fortunate few who may object to the pedanticism of the following discussion.

The Mean as a Measure of Type

The human mind is so accustomed to the use of averages that it seems superfluous to discuss that representation of the value of a series of measurements which differ among themselves; and yet, as is not infrequently the case with the commonplace, the philosophical implications associated with that constant are but rarely considered. It is the function of the mean to provide a primary characteristic value which, in a broad sense, summarizes the individual magnitudes in a readily comprehensible single quantity. In it *every* measurement comprising the series is *equally* represented. These individual measurements or observations are referred to by statisticians as *variates*, and the character being studied as the *variable*. Thus the mean, in summarizing the variates, becomes a typical measure of the variable.

Of the methods available for the calculation of the mean, it is entirely adequate for the present purpose to mention briefly two methods that will be of importance in the discussions that follow, perhaps mainly on account of the symbolism they introduce, for symbols play a most important role in the economy of human thought, permitting the expression of ideas with the maximum of clarity, conciseness and precision.

The variable, be it weight per bushel of wheat, diastatic activity of flour, or any other quantity which varies in magnitude from sample to sample, may be represented by any chosen char-

acter. Let x represent any variable. Then $x_1, x_2, x_3 \ldots . x_N$ may be taken to designate the N individual variates which comprise the series. If a macron placed above the symbol for the variable be employed to represent the average value of the variable, clearly

$$\bar{x} = \frac{1}{N}(x_1 + x_2 + x_3 + \ldots . + x_N)$$

or, if the Greek character sigma in the upper case, Σ, be employed to designate summation of all the values of the variable indicated in parentheses

$$\bar{x} = \frac{\Sigma(x)}{N} \qquad \text{(i)}$$

Not infrequently the values of x are large numbers and the summation of a lengthy series of such measurements without the aid of a mechanical computing device presents difficulty. In such circumstances one may find it helpful to subtract a constant number from all the values of x and simply average the residuals. This is what is termed by the biometrician "taking moments about an arbitrary origin." Comparable with the terminology of the physicist, a *moment* in statistics simply means the influence of an individual or a group of individuals of a certain numerical value with regard to another number on the same scale. Thus x is the moment of x about zero as origin; $(x\text{-}x_o)$ is the moment of x about the arbitrary origin x_o; $(x\text{-}\bar{x})$ is the moment of x about its mean as origin. These are all first moments. The squares of these quantities would be the second moments respectively, the cubes the third moments and so forth. Employing moments about an arbitrary origin, one may write the expression

$$\bar{x} = x_o + \frac{\Sigma(x\text{-}x_o)}{N} \qquad \text{(ii)}$$

For the greatest reduction of labor in computation, the value of the arbitrary origin x_o is usually selected as a round number approximately equal to the average of the series. On this account some workers refer to it as "the guess at the mean."

The inadequacy of $\bar{x}$ as a complete description of the variable will be apparent to every intelligent worker, for it obliterates certain of the most salient features of the series of variates. If one may term the mean as the characteristic magnitude of the series, for a clear description of the variable it remains a primary necessity for the student to measure the distribution of the individual measurements about this characteristic magnitude.

Deviation From Type

Variation must be measured with regard to some fixed point of reference. There surely can be no more logical origin for the establishment of deviations among a series of measurements than $\bar{x}$, the value typical of the series. Hence the deviation from type of each variate may be expressed as $(x-\bar{x})$, or the first moment of x about its mean. Obviously, to average these N values for the series in order to obtain a single measure of variability for the system would be of no avail, for by the definition of $\bar{x}$,

$$\Sigma(x-\bar{x}) = 0$$

The difficulty of positive and negative deviations that just balance one another may be removed in either of two simple ways. First, the deviations may be summed without regard to sign, and the total divided by N to secure the *average deviation*. Second, the deviations may be squared to give quantities of positive sign throughout. If the square root of the average of these second moments about the mean be taken, the resultant quantity will be again a linear dimension. This latter quantity is known as the *standard deviation*. The almost universal use of the standard deviation in preference to the average deviation as a measure of variation in absolute terms, despite the greater ease of comprehension of the latter, is due to the tremendously greater serviceability of the former in arriving at simple formulas for the expression of more advanced concepts. If the standard deviation were to be replaced by the first moment function for the measurement of variability, several decades of notable achievement in statistical analysis would have to be laid aside for a less fruitful attack upon the many problems which already have been solved by its use.

The lower case Greek character sigma, σ, has become universally employed to denote the standard deviation, with a defining subscript to indicate the variable referred to. Accordingly the equation will be given as

$$\sigma_x = \sqrt{\frac{\Sigma(x-\bar{x})^2}{N}} \qquad \text{(iii)}$$

While this is a most elegant expression in that it shows at a glance the nature of the constant, it is ill-suited for practical application in that cumbersome quantities result for $(x-\bar{x})$ if any degree of precision in computation, necessitating several places of decimals for $\bar{x}$, is observed. For machine calculation, Harris (1910) sug-

gested the equivalent and far more serviceable form employing moments about zero as origin:

$$\sigma_x = \sqrt{\frac{\Sigma(x^2)}{N} - (\bar{x})^2} \quad \text{(iv)}$$

In the absence of a mechanical computing device, the labor of calculation may be greatly reduced by employing moments about an arbitrary origin, using the well known formula:

$$\sigma_x = \sqrt{\frac{\Sigma[(x-x_0)^2]}{N} - (\bar{x}-x_0)^2} \quad \text{(v)}$$

For the comparison of the variabilities existent in different series, it is often helpful to express variation on a relative scale. For example, the standard deviation of replicate protein determinations in a critical study of several workers employing a standardized technique (Treloar, 1929) is approximately .122% protein, whereas in a more general study it has been demonstrated (Treloar and Harris, 1928) that the standard deviation of replicate ash determinations is of the order of .009% ash. It is not possible to compare these variabilities directly because they are on an absolute scale. If, however, they be expressed as percentages of the respective average values, becoming 0.8% for protein and 1.7% for ash, it is at once apparent on this readily comprehensible basis that protein is at present the more precise determination. This *relative* measure of deviation is known as the *coefficient of variation*, where

$$C.V \text{ (or } V) = 100\frac{\sigma_x}{\bar{x}}\% \quad \text{(vi)}$$

A word of warning may not be out of place in reference to this constant. In its interpretation caution must always be exercised not to neglect the fact that its magnitude is partly a function of $\bar{x}$. To compare by means of the coefficient of variation the consistency of replicated protein determinations with that of the comparable "blank" determinations will give an entirely fallacious representation of the information desired. In this case the values of σ_x should be directly compared.

Two other measures of variation of more limited service in studying the distribution of variates may be mentioned. These are *range* and *probable error*. The range simply expresses the deviation between the minimum and maximum values of the variable. It has very little value when applied to the sample because of the

large errors of random sampling attaching to these extreme values. Moreover, these minimum and maximum values of the variable are not infrequently determined solely by two or at best very few variates. The weakness of the logic that would measure variation in the entire system solely by these magnitudes is sufficiently apparent of itself. Every variate is of importance in determining variability just as in the establishment of the average value.

The probable error is that deviation on either side of the mean which embraces 25% of the variates in each direction. It is but infrequently used in connection with original variates, although reference is sometimes made to the probable error of a single determination. This latter value gives the range within which the central 50% of observations may be expected to fall, usually on the assumption that the distribution of the variates follows the normal curve of error. It is arrived at by the theoretical procedure of multiplying the standard deviation by the factor .6745.

Insomuch as the probable error has conventionally been employed in connection with the variation of statistical constants, further attention will be given this measure in a following section of this review, but it may be mentioned here that its use is largely due to the persistence of convention and not to any added usefulness over the standard deviation from which it is derived.

The Distribution of Frequency

That fundamental law of nature which makes it possible for us to understand probability, the law which recognizes that variation is not chaotic but follows well defined systems, need not be emphasized to workers in the field of cereal chemistry. It is manifest whenever like values in a reasonably large series of measurements are accumulated in an orderly fashion. While the mean and standard deviation are necessary for the precise measurement of type and deviation from type, the graphical presentation of the frequency distribution involved remains invaluable in clearly presenting the differences in two or more series of measurements. It is not out of place, therefore, to discuss briefly some of the types of frequency distribution, particularly as the subject is so pertinent to an understanding of the reliability of individual measurements and of statistical constants.

One may perhaps be permitted for clarity of presentation to center this discussion about a few typical examples from published records of cereal data. Bailey and LeVesconte (1924), in a study of the serviceability of the Chopin extensimeter, made 10

determinations on each of 25 replicate doughs prepared from the same flour. The 250 measurements, when seriated into classes, form a frequency distribution that is graphically presented as the histogram in Figure 1. The heights of the rectangles give the number or *frequency* of the tests that gave results falling between the limits demarked on the base scale by the vertical sides of the column in each case. Grouping into classes of greater range than the smallest unit of original measurement is necessary because with the finer classes the frequencies in each would be so small, and the errors of random sampling so magnified, that the true nature of the curve would be considerably obscured. Speaking in terms now of the *unit of grouping* employed in the graph, which in this case is one unit of "extensibility," the area of the entire set of columns gives the total number of tests represented, whereas the

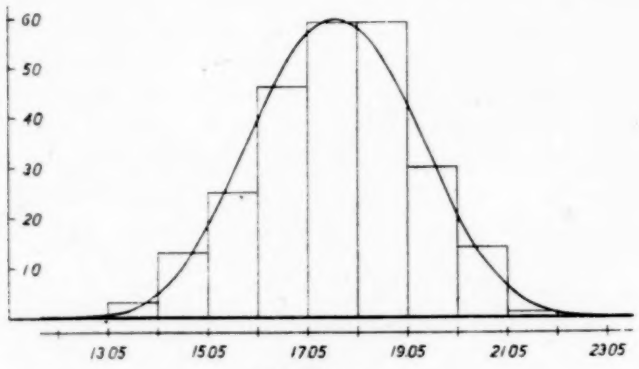

FIGURE 1

Histogram of the distribution of 250 determinations of extensibility of 25 replicate doughs prepared from the same flour (Bailey and LeVesconte, 1924), with the fitted curve of frequency superimposed

area between any two vertical rules gives the number of tests falling between those limits. The ratio of the latter to the former will then give, according to the sample itself, the probability of tests falling within the designated range. Specifically, the probability of obtaining extensibility values for these doughs below 14.1 units is 3/250 = .012, or 1.2%.

As a basis of generalizations, the histogram above has very definite limitations due to the relatively small number of tests it represents and the coarse grouping that consequently has had to be employed. If a very large number of tests were available, so that a unit of grouping equal to 1/10th of a unit of extensibility could be employed, each of the rectangles in Figure 1 would be split into 10 rectangles, not all of the same height as portrayed in

the figure above, but changing in a systematic manner from one to the other as do the coarse groups in the present sample. If an infinite number of tests were available, it is most likely that, with the infinitely fine grouping then possible, a distribution would be obtained of the type given by the flowing line in Figure 1. This, then, is the purpose of curve fitting: to determine from the sample by statistical procedures the most likely form of the distribution if an infinitely large number of individual measures of the variable were available. Insofar as it is possible to make valid deductions from the sample, one should employ the curve instead of the histogram. Thus the probability that values for extensibility up to 14 units will be obtained becomes corrected to a value of $2/250 = 0.8\%$.

This discussion may not attempt the task of detailing the methods to be employed for curve fitting. The enquiring reader may be referred to the brief abstract by Elderton (1906) of the fundamental memoirs of Karl Pearson for satiation of his desire. However, brief reference must be made to a very fundamental curve of statistics, namely the "normal" curve, and the general characteristics of some other common types of distribution.

Frequency systems may be broadly divided into two groups, the *symmetrical* and *skew* distributions of the statistician. Figure 1 belongs to the former group, wherein positive and negative deviations from the mean are equally balanced. In the skew distribution this balance of frequencies about the mean is not realized, the peak of the curve being "pushed off center" so to speak. The point of greatest frequency is known as the *mode*, and hence various functions of the separation of the mean and the mode are employed as measures of *skewness*. Within these groups the frequency distributions are classified chiefly according to whether the curves end abruptly on the base line on one or both sides of the mean, or approach the base line as an asymptote; i. e., according to whether the range be limited or infinite in either or both directions. According to this scheme Pearson has classified the distribution of frequency into seven types and provided criteria based on the first four moments of the systems to enable workers to identify the types and fit the curves to observational data.

Mangels (1927) has classified the distribution of protein in three crops of North Dakota H.R.S. wheat according to this Pearsonian series, but did not fit the curves. In Figure 2 the histogram and fitted curve for the distribution of protein content in 546 samples of H.R.S. wheat according to the data presented by Thomas (1917) is presented. This system illustrates a skew distribution of

limited range in the direction of low protein but unlimited with regard to high protein. Here the mode occurs at the value 12.5% protein, while the mean is 13.85%. The standard deviation (1.53%), measuring the dispersal of the system, does not lead to a definite pair of points on the curve as in the case of the "normal" distribution.

There is not any curve of frequency distribution of greater service for the smoothing of variable measurements or the representation of the distribution of replicated constants, than that known as the normal curve of error. De Moivre, in 1733, estab-

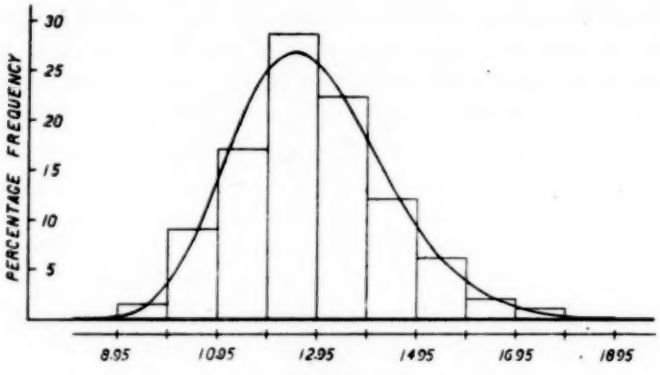

FIGURE 2

Histogram for the distribution of percentage protein in 546 samples of H. R. S. wheat, according to the data of Thomas (1917). The fitted curve belongs to Type III of the Pearsonian series.

lished this law of the distribution of chance variation under the influence of the intense interest of the 18th century nobility and gentry of England in the outcome of games of chance. It is given by the expansion of the binomial $(a + b)^n$ when $a = b$ and n is infinity. De Moivre's work lay buried, the credit being given to the subsequent "discoverers" La Place and Gauss, until quite recent times (Pearson, 1924), so that the curve is still frequently referred to under the names of the latter workers. It is a symmetrical curve of theoretically infinite range, and portrays the distribution of a variable when chance alone, composed of equal probability for and against the event, determines the variation. Outside of its tremendous importance in establishing the distribution of many fundamental constants to be expected from random sampling, it provides the equation for smoothing many simple variables. A typical biological case is provided in the stature of human adults. In the field of cereal chemistry an interesting example is found in the distribution of loaf volume when the influence of protein content of the

flour is removed (Treloar and Larmour, 1931; Treloar, Harris, Sherwood and Bailey, 1931). The fundamental significance and usefulness of the normal curve both in the biological and social sciences as well as in theoretical statistics has led to the preparation of tables (notably by Sheppard 1901,—republished by Pearson, 1924) to enable workers to readily apply it to actual data without onerous calculation.

In order to completely define the normal curve smoothing any series of observations or deductions, it is merely necessary to know the mean and standard deviation of the series; the former fixes the position of the curve on the scale of dimensions, and the latter determines its spread, for the standard deviation is the distance from the central axis (the mean) to the point of inflection of the normal curve. Although theoretically infinite in range, the curve becomes essentially a part of the base line at a distance on either side of the mean equal to three times the standard deviation.

On account of the ease of application of the normal curve of error, theoretical deductions are frequently made from it on the assumption, which must be philosophically sound, that it represents the form of the data under consideration. This is justifiable only in those cases where the data is too scant to permit of the intelligent application of mathematical criteria to establish the true curve form.

Now it is not illogical, in the absence of more accurate studies of human bias in chemical analysis, to believe that the errors made by a careful analyst in replicating the protein determination on a uniform sample of flour, will be of a random nature that is attributable to the combination of chance factors. That is, the distribution of the analyses may be expected to accord with the normal curve of error. If sufficient determinations are available to enable the calculation of a dependable standard deviation, the curve of error may be drawn and from it the probabilities determined (as indicated previously) that errors between any given limits will occur. Initial work of this type has been undertaken by the Association's Committee on Methods of Analysis (Treloar, 1929). The curve obtained therein was based upon the average standard deviation of 20 replicates by each of several workers, and the position of occurrence of ordinates marking off the central 50%, 80% and 99% was determined by obtaining the probable error of a single determination to establish the first mentioned zone, and then multiplying this value by 1.9 and 3.8 respectively for the other

two values, the ordinates being erected on either side of the mean to demark the respective zones of probability.

The above method of establishing probability is equally applicable to all types of frequency distribution, with this reservation, of course, that different numerical factors to those above will be needed in non-normal curves.

Too much stress cannot be laid upon the necessity of obtaining the correct graduation of the system involved if the deductions are to be accepted as valid generalizations. A fundamental problem of the biometrician in practical issues is to convince the worker, whether he be biologist, bio-chemist, bio-physicist, or social scientist, of the dangers of drawing conclusions from too few observations, from which it is often impossible to know the extent and type of variation that must be interpreted. Tremendous sums of money are being wasted annually through the fervor of investigators for extensive rather than intensive research, and cereal chemistry is not entirely free of the criticism.

The Concordance or Disagreement of Observation and Theory

In variation studies one is not infrequently confronted with the situation as to whether an observed distribution differs only by the errors of random sampling from a theoretical distribution known, perhaps, *a priori;* or whether two or more observed distributions that differ among themselves could have arisen as random samples from one general population. The geneticist frequently faces the problem as to whether segregating generations agree in their frequency proportions with a known type of Mendelian ratio, or the cereal chemist wishes to decide whether there is essential agreement between a variable series of analyses and some expected curve or another series of analogous determinations. The methods of approach to the solution of such problems must fall into two general classes. First, the distributions must be directly compared in themselves; or second, constants derived from the distributions must be contrasted in relation to their probable errors. While a little may be said in reference to the second category of methods later in this discussion, it is the present purpose to direct the reader's attention to the powerful tool of analysis under the first head provided by a monumental work of Pearson (1900). This is known as the χ^2, P criterion, by means of which it is possible to determine very simply with the aid of Elderton's tables (1901—republished by Pearson, 1925) the probability that the existing differences or greater ones could have arisen through the errors of

random sampling alone. If O be the observed frequency in any class of the variable, C the corresponding theoretical frequency, then the sum of the entire series of quantities $(O-C)^2/C$ gives the χ^2 value.

$$\chi^2 = \Sigma \frac{(O-C)^2}{C} \qquad \text{(vii)}$$

The corresponding probability that the discrepancy is merely due to chance is determinable by interpolation in Elderton's tables.

In an extension of this method, Pearson (1911) has considered the case where two observed distributions are to be compared as having possibly both originated from the same, but unknown, population of measurements. The formula for χ^2, for which the reader is referred to the original paper, is quite similar, and P, the probability of common origin of the two, is obtainable in like manner.

Before considering the second category of methods of comparison, it may be well to devote some thought to the general problem of the variation of statistical constants.

The Probable Error Concept

It is a simple philosophy that the trustworthiness of any statistical constant must be a function, in part at least, of the variability of the original measurements and the number of them upon which the constant is based. And yet a clear grasp of this dictum will go a long way toward clarifying to the lay mind the whole concept of probable errors, for these latter quantities are simply the needed functions as provided by the mathematical statistician. There is no need for the biological worker to attempt to follow the oft-times intricate processes of mathematical reasoning that lead to the establishment of these functions. There is, however, weighty reason why he should intelligently grasp the interpretation of probable errors if he is to lay claim to sound processes of logic in his deductions from variable data.

The probable error concept flows very smoothly from a understanding of the general principles of frequency distributions already briefly discussed in these pages; for any statistical constant determined from a sample is simply one member of a series of like constants which would arise if the experiment were replicated. These constants, upon seriation, would comprise a smooth distribution. The drawing of conclusions would be very much simplified if it were possible to replicate the constant a sufficient number of

times to fully establish the position in space and standard deviation of its frequency curve. However, there are economic limitations to most research programs, and not infrequently the worker must be content with the information provided by the single constant and its probable error. This probable error value is simply the best measure available under the circumstances to define the spread of the frequency distribution to which the constant belongs. It is comparable to the standard deviation of the replicated constants if they were available, except that it is just .6744898. . . of that standard deviation, and is theoretically arrived at from the knowledge of the distribution of the original variates. Indeed, the old convention of multiplying by this factor (.6745) to give the zones of equal probability on the distribution curve (a habit adopted from a practice of the astronomers), is now being dropped, but a compromise is being made by using the term *standard error* to mean the standard deviation of the curve of replicated constants. Thus, if k be any constant whose distribution follows the normal curve of error.

$$P.E._k = .6744898\sigma_k$$

This accounts for the constant occurrence of this numerical factor in probable error formulas, the standard deviation (or standard error) being theoretically deduced first and then reduced to the form "probable error."

No attempt is being made in these pages to detail all the considerations which came into the establishment of probable error formulas. It may, however, be stated that if the original variates are distributed in the form of a normal curve the commonly used formulas:

$$P.E._{\bar{x}} = .6745\ \sigma_x/\sqrt{N}$$

$$P.E._{\sigma_x} = .6745\ \sigma_x/\sqrt{2N}$$

may be rigidly employed. If the frequency distribution of the variates differs widely from the normal curve, the formula for $P.E._x$ may still be applied with safety if x is based upon 20 or more determinations ("Sophister," 1928), but that for $P.E._{\sigma_x}$ must be determined from the more precise formula[1] (Pearson, 1903).

$$P.E._{\sigma_x} = .6745\sqrt{\frac{\mu_4 - (\mu_2)^2}{4N\mu_2}}$$

[1] For illustration of the differences involved for $P.E._{\sigma_x}$ with non-normal distribution, the reader may be referred to the practical illustrations given by Harris and Ness (1928).

where μ_2 and μ_4 are respectively the average second and fourth moments of the variates about the mean as origin.

Returning for illustration to the data on extensibility of Bailey and LeVesconte presented graphically in Figure 1, it is possible to draw the curve for averages of *random* samples of 10 determinations from knowledge of the mean and standard deviation of the original variates, for the mean of the new curve will be the same as that of the original series, and the standard error will be $(1/10)^{1/2}$th part of the original σ_x. The curve has been drawn and is

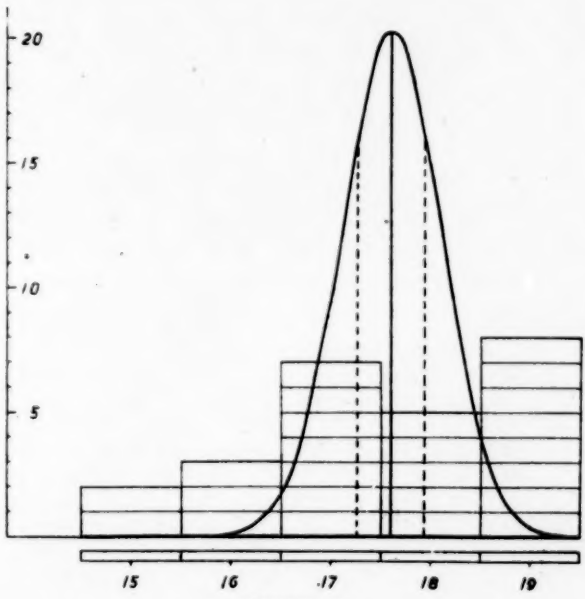

FIGURE 3

Theoretical curve for the distribution of averages of 10 extensibility measurements selected at random from the data of Bailey and LeVesconte (1924). The histogram gives the actual distribution of averages of 10 trials each on 25 replicate doughs.

presented as the flowing line in Figure 3. It appears in this curve that if the doughs be truly undifferentiated, the conclusion may be drawn that the averages of 10 replicates may be expected to vary over a range from approximately 16.0 to 19.2 with 17.6 (the mean of the series) as the most likely value. If on this curve at a distance equal to the probable error on either side of the mean perpendiculars (given by the heavy broken lines) be erected, one-half of the area of the curve will lie between these perpendiculars, and the other half equally divided between the two "tails" of the curve. If the 25 replicate doughs studied by the above authors differed

from one another only in the same manner that repeated tests on any single dough differed among themselves, then averages of the 10 extensibility measurements for each of the 25 doughs should follow the above curve. It is of interest to make this comparison, and accordingly the histogram for the 25 averages has been superimposed in Figure 3, where very broad grouping has necessarily been employed. It is entirely obvious that observation is not in agreement with theory in this case, and the conclusion may safely be drawn that the discrepancy could not have arisen through the errors of random sampling without appeal to the χ^2, P criterion. The authors' contention on the grounds of analagous experience that replicate doughs from the same flour would be differentiated on account of the difficulty of duplicating "all the conditions that effect the properties of a substance as sensitive to treatments as such material" is entirely borne out in fact by the submitted results.

Had only 10 determinations been available in the first instance, the picture would not have been as clear. One would have had to rely upon the standard deviation of the 10 for a measure of the distribution of the variates, and would not have known whether the single average was equal to the true mean of the large series or not. Speaking perhaps loosely for a moment, one may say that the probability of occurrence of any particular value within the range of a curve is proportional to the height of the curve above that point. Obviously the mode is the most likely value. In the absence of further information it is entirely logical to assume that the single value obtained of the constant is the mode (which is the true mean in the normal curve) since that value is the most likely one to occur. Thus one theoretically erects a curve about the single value obtained as center, expressing its spread by obtaining the probable error of that mean according to the formula provided. If one was fortunate enough to obtain an average that was equal to the true mean the picture would be sound and precise conclusions could be drawn. If, however, the single average of 10 happened to be one that was not likely to occur very often, i.e., approaching the extreme values as noted in Figure 3, the picture would be quite erroneous. But, *in the absence of further information*, and employing the logic above, conclusions are usually drawn on the assumption that the single constant is the true value. This point of view is stressed here to emphasize the desirability of working with a sufficiently large number of individuals so that the constants obtained will be highly trustworthy; or, in other words, so that the possible variation will be small and hence any error introduced by

the above assumption proportionately lessened. It is well to remember that individual constants based on the same number of observations and with approximately the same *P.E.* may be as far apart as 7.2 times that *P.E.* and belong to the same distribution! This, however, will only occur about once in one hundred times. In like manner there is a definite probability attaching to every difference between comparable constants.

The Significance of Statistical Constants

It is a trite statement that, in this universe, nothing is great or small except by comparison; yet it is not infrequently forgotten that this is to state that no magnitude has significance in itself unless it involves a comparison. To blindly apply a statistical criterion that a constant may be considered significant if it is so many times its probable error will therefore lead to false impressions, for a mean or a standard deviation is a simple magnitude and can have no significance of itself. The criterion becomes valid at once, however, if reference be made to the difference of two constants.

Referring again to the extensibility study of Bailey and LeVesconte (1924), it is apparent that the 25 means for the several doughs offer opportunity for the making of 300 different comparisons between individual pairs of these constants. These comparisons may be expressed as differences, and once again (after seriation) a frequency curve will be found to smooth the distribution obtained. Assuming that the differences are determined by always subtracting one mean from the other in a definite order, the average of these positive and negative differences must equal zero. Now if for purposes of the present discussion, thought be directed to the fact that the 25 doughs have originated from the same flour sample and are true replicates in that sense, the distribution curve for zero differences in extensibility would be established by actual test. If, from another flour of unknown origin, an average extensimeter reading was obtained and it was compared with any selected average from the first series, then if the difference should fall within the above curve it could not be said that the two flours differed in extensibility of their doughs. If, however, the mean for the new flour fell outside the practical range of the curve for the first flour, differentiation between the two in dough extensibility would be considered to be established.

Again by mathematical reasoning, the biometrician has to attempt to arrive at a knowledge of this curve of differences when

he merely has at his command the two single constants and their probable errors. It can be demonstrated that, under the more common conditions of biological work, this curve is also of the normal type, and that its standard deviation is given by the square root of the sum of the squares of the standard deviations of the two constants. Now since the probable error is a constant fraction of the standard deviation on the normal curve, it follows that

$$P.E._{(a-b)} = \sqrt{P.E._{a}^{2} + P.E._{b}^{2}} \quad \text{(viii)}$$

where a and b are two comparable constants. It has already been indicated that a value distant from the mean greater than 3.8

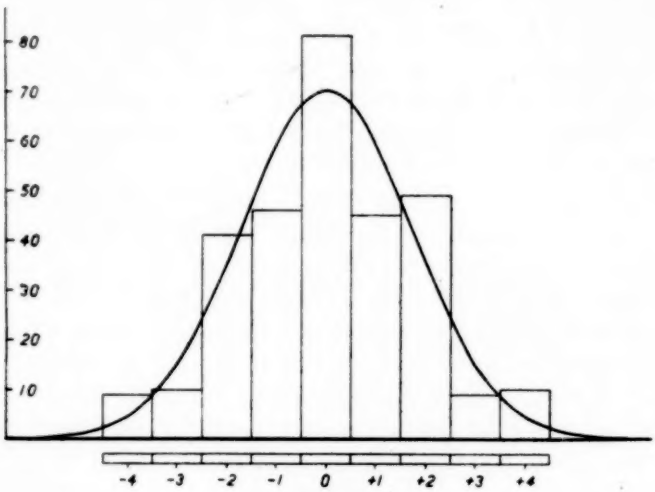

FIGURE 4

Histogram and theoretical curve for the distribution of the 300 individual differences between 25 average values for the extensibility of a like number of replicate doughs (data from Bailey and LeVesconte, 1924)

Employing equation (viii), Figure 4 presents the theoretical curve of expected differences in extensibility between doughs prepared from the same flour, and the histogram for the 300 actual differences as calculated from the data given. Its chief purpose is to emphasize the range of differences in the means that might be expected to occur with doughs prepared from the same flour. times the *P.E.* falls outside of the practical range of the normal curve. Thus a difference between two constants greater than 3.8 times its own probable error is essentially different from the mean of that curve, and therefore has a significantly different magnitude.

Without this knowledge it would be idle to attempt to conclude whether two flours of fairly close extensibility were really differentiated in this respect.

A tremendous amount of work in theoretical statistics yet remains to be done upon the significance of differences between constants. What is stated above may be accepted as well established in application to the difference in average values, provided no correlation exists between the variates of the two series from which the constants are obtained. Where correlation does exist, correction must be made for it, but this is a matter which must be reserved for the next discussion in this series.

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REPORT OF THE 1930 MEETING OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

J. A. LeClerc, *General Referee*

At the meeting of the A. O. A. C., held in Washington, October 1930, the following reports of collaborative work were submitted by the various associate referees:

a—H. Runkel—Methods of sampling flour.

b—M. J. Blish—Glutenin.

c—L. H. Bailey—Determination of starch in flour.

d—L. H. Bailey—Methods for sampling and determination of moisture in Bread.

e—G. C. Spencer—Flour Bleaching Chemicals.

f—J. H. Bornmann—Lipoids and Fat in Baked Products.

g—S. C. Rowe—Organic and Ammoniacal Nitrogen in air-dried baked products.

h— ———— Moisture in Alimentary Pastes.

i— ———— Collecting and Preparing Samples of Alimentary pastes for Analysis.

j—R. L. Horst—Crude fiber in Alimentary Pastes and in air dried baked products.

k—S. Alfend—Unsaponified matter in flour and in Alimentary Pastes.

l—M. J. Blish—Experimental Baking Tests.

The following recommendations were made by the General Referee and adopted by the Association:

Flour

(1) That the tentative method (Official, 1st Action) of sampling flour be made Official, Final Action and that further collaborative work be discontinued for the present.

(2) That until the chief protein constituents of wheat flour are reliably identified and correlated with flour properties or characteristics, attempts to develop methods for the quantitative determination of glutenin be discontinued.

(3) That further collaborative work be carried on with the tentative (Rask) method of determining starch, and that it be compared with the more recent modification of the Hartmann and Hillig method.

(4) That the tentative Seidenberg method for determining chlorine in chlorine-bleached flour be again studied and compared

with a modification thereof which consists of adding ammonium or sodium bicarbonate to the ether extract, and that studies be made for the detection of benzoic acid in flours treated with organic peroxides containing a benzoyl radicle.

(5) That special (non-collaborative) studies be continued of the methods for unsaponifiable matter in flour, alimentary pastes and baked products in conjunction with the same study on eggs.

(6) That the study of rapid methods of ashing flour, bread, and other baked products be further made. That the study of the nature of the losses which take place on fusion of ash be temporarily discontinued.

(7) That a comparative study be made of the colorimetric and quinhydrone electrode methods of determining the hydrogen-ion concentration in order to determine the applicability of the colorimetric method to cereal products.

(8) That studies be made of methods of estimating the diastatic value of flour and of its effect on the baking value of the flour.

(9) That comparative tests be made of foreign and domestic methods of chemical analysis used as a measure of determining the value of the flour.

(10) That the present tentative method for water soluble protein nitrogen precipitable by 40% alcohol be dropped and that in its stead the proposed tentative method for "Albumin Nitrogen" be adopted. This method reads as follows:

> Place 20 grams of the sample in an 8-ounce nursing bottle, add 100 cc. of water fom a pipet, shake the bottle to prevent lumping of the sample, and add exactly 100 cc. more of water. Mix the contents of the stoppered bottle gently by hand or on a slowly revolving wheel for 1 hour. (The temperature of the water should not exceed 30° C.) Centrifuge to facilitate filtration and filter through a thin asbestos pad in a Hirsch funnel, using light suction. Determine nitrogen in 50 cc. of the filtrate as directed in the official method[2] distilling the ammonia into 20 cc. of 0.1 N acid. Run a blank on the reagents. Pipet off 100 cc. of the above filtrate into a 200 cc. volumetric flask, add 15 cc. of sodium chloride solution (28 grams diluted to 300 cc.), fill nearly to the mark with 95% alcohol, mix carefully to avoid foaming, cool to room temperature, make up to the mark with alcohol, mix well and allow to stand overnight. Pipet off the supernatant liquid and filter through an 18½ cm. fluted filter paper. Determine nitrogen in 100 cc. of the filtrate as above. (In order to avoid bumping, it is advisable to add the sulfuric acid and boil off the alcohol before adding the potassium sulfate and mercuric oxide.) Subtract the value obtained from the water-soluble nitrogen to obtain the albumin nitrogen.

(11) That methods for the determination of O_2 in self-rising flour be studied.

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[2] Methods of Analysis. A. O. A. C. 22: 8 (1925).

Alimentary Pastes

(1) That the tentative method for the determination of moisture in alimentary pastes be further studied.

(2) That the (Official, 1st Action) method adopted officially for the determination of crude fiber in flour be further studied to determine its applicability to alimentary pastes and bread.

(3) That the tentative (Official, 1st Action) method for fat by acid hydrolysis in alimentary pastes be further studied.

(4) That the tentative (Official, 1st Action) method for lipoids and lipoid phosphoric acid in alimentary pastes be studied collaboratively in comparison with the chloroform-alcohol extraction method suggested by Mitchell.

(5) That the present tentative method of collecting and preparing a sample of alimentary paste for analysis be dropped and that the following be substituted therefor:

> Pick out sufficient strips from the lot to be analyzed to insure a representative sample. Break these by hand into small pieces. Mix and grind 300-500 grams in a mill until all of the material passes through a 20-mesh sieve. Keep in a sealed container to prevent changes in moisture content.

(6) That in Method I for "Total Solids and Moisture" (indirect method) delete everything after the 1st paragraph and as thus modified the method be made Tentative.

Bread and Baked Products

(1) That a study be made of methods to determine milk solids in bread.

(2) That a study be made of methods to determine rye in bread.

(3) That the (Official, 1st Action) method to determine chlorides in baked products be studied for the purpose of making this method Official, Final Action.

(4) That the tentative Standard Baking Test be continued as such.

(5) That the tentative method for the determination of moisture in bread be made Official, Final Action.

(6) That the tentative method (Official, 1st Action) for the determination of moisture in cake be further studied.

(7) That the method for total solids in air-dried bread by heating at 130° C. for one hour be further studied.

(8) That for the purpose of making the usual chemical determinations in bread one half of the loaf be taken as a sample. (Official, 1st Action.)

(9) That the tentative method for determining fat in bread by acid hydrolysis be made Official, 1st Action.

(10) That further study be made of the method to determine lipoids in baking products.

(11) That the tentative method (Official, 1st Action) for the determination of organic ammoniacal nitrogen in baked products be made Official, Final Action, and that for this purpose the Gunning and the Kjeldahl-Gunning-Arnold Methods be given preference.

BOOK REVIEWS

Milling and Baking Qualities of World Wheats—By D. A. Coleman, et al. 223 pages. U. S. Dept. Agr. Technical Bulletin 197. For sale by Superintendent of Documents, Government Printing Office, Washington, D. C. Price 35c.

U. S. Department of Agriculture Bulletin 197 is a compilation and discussion of milling, baking, and chemical data obtained in examining wheats produced in most of the principal wheat-growing areas of the world. The bulletin contains considerable information of interest to cereal technologists and grain experts.

Two general types of samples were used for study—namely, varietal samples and export samples. In case of varietal samples, the attempt is made to secure data on representative samples of all types and varieties produced in the particular area. Most of the export samples were obtained from the principal wheat exporting areas of the world, namely, Canada, United States, Argentina, and Australia. Data is presented for wheat from practically every producing country of the world, except Algeria, China, France, Jugo-Slavia, and Roumania. Data on acreage and production in the different countries is included.

The bulletin discusses wheat quality from a bread-making standpoint only, and does not consider the use of durums and soft wheats for paste and pastry products. With regard to presentation of data the authors make this statement:

"It is assumed that most of the wheats grown throughout the world are ground into flour for bread-making purposes; therefore, in estimating quality in addition to milling quality their utility for bread-making purposes has been used as the yard stick of quality. Further, the ability of the various flours to make bread that suits the American standards of bread-baking has been used as the basis of quality throughout. It is conceded that some of the wheats that prove inferior under this system of evaluation might make acceptable products if a different standard of baking quality were used."

The samples of wheat were graded according to the Official Grain Standards of the U. S. Department of Agriculture. The milling and baking procedures are described and the chemical determinations were made in accordance with the "Methods of Analysis" of the American Association of Cereal Chemists. The samples of wheat were milled to a "straight grade" flour on an experimental mill. Milling data presented includes yields of flour, and notations regarding milling characteristics of the wheat and the texture of the flour obtained. The baking procedure was a straight dough method using 340 grams of flour. The fermentation and proofing time were variable, depending upon the type of flour. The baking data includes time of fermentation and proofing, absorption, loaf volume, weight, and other description of loaf characteristics.

Considerable chemical data is presented, but the data are not adequately

discussed. Chemical data includes protein, ash, pH, titratable acidity, gasoline color value, glutenin, gliadin, and the Gortner g.uten quality index. One very informative chemical determination, unfortunately, has been omitted, namely, the diastatic activity of the flour.

The wheats are grouped according to continents in the following order: North America, South America, Europe, Asia, Africa, and Oceania. Within continents, countries are arranged alphabetically.

North America—For North America data is presented on wheats from Canada, Mexico and the United States. The bulk of the Canadian crop consists of hard red spring wheat which averages high in bread-making qua.ity. The Mexican wheats are low in protein and of inferior baking quality.

The various types of wheat produced in the United States are represented. From standpoint of baking quality the Hard Red Spring wheats of the United States were of superior quality, followed by Hard Red Winter types. The authors consider the white wheats as being inferior to Hard Spring and Hard winter in baking quality, but superior to soft red winter wheats.

South America—Wheats from Argentina, Chile, and Uruguay were examined. Argentina wheat, being an important competitor of United States wheat in the world market is of especial interest. The data presented indicates that the North American hard winter wheats are slightly superior to Argentina hard winter types in baking quality. The baking quality of soft winter types produced in Argentina compares favorably with the same type produced in the United States.

Europe—Wheats were secured from Belgium, Bulgaria, Czecho-Slovakia, Denmark, England, Austria, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Norway, Poland, Russia, Scotland, Spain, Portugal, Sweden, and Switzerland. With the exception of Russia, all of the above countries produce wheats which would be considered as inferior in baking strength. The authors consider the Russian Hard Red Winter as lacking in baking strength as compared to United States wheat of the same type. The Russian durums show a comparatively high baking strength and were the only durums which equalled the North American durums in this respect.

Africa—Wheats were secured from Egypt, Morocco, Tunis and South Africa. None of these areas produced wheat of high bread-making quality.

Asia—Wheats were secured from India, Iraq, Japan, and Palestine. India is the most important producer. The authors consider the Indian white wheats as inferior in baking quality to those of North America and Australia, and further state that "apparently Indian wheats should be blended with other wheats to obtain the best results." A sample of Marquis from India showed very good baking quality.

Oceania—Wheats were secured from Australia and New Zealand. New Zealand wheats lack strength. Australian wheats are mostly common white wheat of winter habit and are of fairly good baking strength. The authors consider Australian white wheats as equivalent to North American white wheats in baking strength.

Considering the large volume of data presented in Bulletin 197 the discussion is rather brief. This criticism applies particularly to the chemical data and to a lesser degree to other data presented. Some unnecessary duplications in data occur, e.g., the milling yield is given both as percentage of flour, and pounds of wheat required for a barrel of flour, and the weight of loaf as well as pounds of bread per barrel of flour.

After a careful reading of Bulletin 197, one cannot help but be impressed with the importance of North America as a producer of high quality bread wheats. The data presented bears an important relation to the future of the wheat growing industry in both the United States and Canada.

—C. E. MANGELS.

Die Theorie der praktischen Brot- und Mehlbereitung. Fifth Edition. 352 pages. Price 8 Marks. F. A. Gunther und Sohn A.-G., Berlin. 1930.

This is the fifth edition of Fornet's manual of practical bread and flour production. The first edition appeared in 1920 and was about one-half the size of the present edition. The latter has been divided into about 53 sections or chapters. The scope of the manual is fairly comprehensive. It begins with cereal production in the world, and closes with milling costs and cost accounting. The space between is occupied by a discussion of most of the details that have been identified with the problem of how to produce superior bread.

Fornet includes a description of the methods employed in the testing of wheat and flour. Several of the testing appliances that he devised are described in this portion of the book. A brief outline of rye and wheat milling practices is also included. Chemical and yeast leavening are next discussed, together with yeast-testing methods. Dough preparation, fermentation, the role of various dough ingredients, bleaching and improving combinations occupy a large section of the manual. Ovens and baking are treated in a highly graphic manner. Grain, flour, and bread enemies, including insects, molds, and rope are next discussed briefly. Then follow certain physical considerations, including thermometry and humidity. Valuation of bread is next presented including measurements of loaf volume, elasticity of crumb, porosity, acidity, color and specific weight. Under "baking tests," Fornet exploits his "Mehlometer" which provides a simple device for fermenting and baking 100 gram doughs in a small pan which is apparently square in horizontal cross-section. Diabetic breads and the nutritive value of bread then receive consideration; this section includes a discussion of vitamins, salts, and the physiological action of baked products.

Like all pandemic treatments of such an expansive subject, the manual fails to provide many details which are of prime concern to the careful student. On the other hand, pandemic text-books have their place, and may be of distinct value, particularly when written by a careful student. In the latter classification Dr. Fornet doubtless belongs. He has accordingly rendered a real service in maintaining his manual in an up-to-the-minute form through these frequent revisions. —C. H. BAILEY.

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